Serotonin and the scientific basis of anti-emetic therapy

Edited by

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Preface

> *It is my belief that this book (sic), representing as it does a truly multidisciplinary approach to this vexatious problem, comes at the beginning of one of the most fruitful periods of research into the topic since the 1950's*

Thus concluded the preface to the book which arose from the 1984 Oxford meeting on the mechanisms and treatment of nausea and vomiting. And so it turned out, although serotonin (or 5-hydroxytryptamine) was scarcely mentioned at the time. Yet the 5-HT_3 receptor antagonists were destined to become perhaps the fastest ever class of new drug to proceed from basic research to clinical use. Within a decade they achieved worldwide prominence with sales of many millions of pounds sterling. But still experience has shown that even they, representing such an enormous step forward in anti-emetic therapy, do not give the complete answer to emetic control, especially outside the areas of cancer chemo- and radiotherapy.

That is why we decided to hold the meeting upon which this current book is based. We felt that there was a need to review the 'serotonin era' in order to consolidate the insights which this approach has brought to our understanding of the whole problem. At the same time we felt it to be important to look to the future so that we might perhaps discover a way forward, something that seemed so elusive in 1984.

This book then is the result of a successful symposium held in Oxford in March 1995 which brought together scientists and clinicians from all over the world. The focus of interest on 5-HT_3 receptor antagonists has brought with it a growth in work on other aspects of serotonin pharmacology and we hope that these are also reflected in the contributions that follow.

At the centre of our endeavour is of course the cancer patient, for whom most importantly, the advent of the 5-HT_3 receptor antagonist anti-emetics has meant a significant increase in quality of life. This has arisen not only directly from superior control of emetic side-effects but also from the increased flexibility clinicians have to use more 'aggressive', and hence potentially more effective, cancer therapy regimens.

Research into the mechanisms and treatment of all types of emesis has come a long way since 1984 when the topic was largely the preserve of a few enthusiastic academic groups. Now it is no longer regarded as rather esoteric but enjoys the attention of numerous talented clinicians and scientists, both in academia and throughout the pharmaceutical industry, many of whom have kindly contributed to this book.

Most of the pioneers of modern thinking in emetic research, like Borison and Brizzee, have now passed on and there has been much re-appraisal of their work in the light of new ideas arising from the development of novel pharmacological tools by these pharmaceutical research groups.

It may well be that, here in the mid-1990's, we are on the threshold of as significant an advance as we were a decade ago. For the sake of our patients let us hope so.

Oxford
December 1995

C.J. Davis OBE
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D.J.M. Reynolds

Chapter 1

Emesis research: a concise history of the critical concepts and experiments

C. J. Davis OBE

" 't is profitable for a man that his stomach should nauseate and reject things that have a loathsome taste or smell"

Robert Boyle, 1627–1691

INTRODUCTION

> Vomiting is perhaps the most complex reflex response which makes extensive use of the autonomic and motor systems. Involved in the reflex activity are salivation, spasmodic respiratory movement effected by the antagonistic action of the inspiratory and expiratory musculature, gastrointestinal reactions of a specialised nature and posture characteristics of the head, body and appendages typically adapted to the process of expulsion of the gastric contents. In addition, there are psychological and cardiovascular effects which fit into the total integrated response (1).

So wrote, in 1980, Shih-Chun Wang who, in conjunction with Herbert Borison in 1950, had published the last major development in ideas concerning the mechanism of vomiting control. They suggested that an emetic chemosensory receptor area existed that was separate from the so-called medullary "vomiting centre" (VC) and designated this the chemoreceptor trigger zone (CTZ) (2). Their major review of the physiology and pharmacology of vomiting published in 1953 began with the words

> The vomiting act is one of the most primitive functions with which animals are endowed. The extreme variety of circumstances under which vomiting can occur defies description. It may follow simple overeating or signal approaching death. It often represents one of the chief signs of drug toxicity regardless of the route by which the drug is administered. In spite of its universal appearance and great clinical importance the nervous mechanism of the vomiting act and the emetic action of many drugs are not well understood (3).

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Despite the passage of 35 years since Wang and Borison's paper and the publication of five thousand scientific papers on this and related topics, little had changed by the time I began working in this area in 1983.

Emesis constitutes a major complaint in a very large number of disorders that are created by virtue of physiological alterations in pathological states. The spectrum of problems that give rise to vomiting is very wide, ranging from normal pregnancy (4) at one end to the bizarre psychopathological aberration of erotic vomiting at the other (5). The complications and consequences of vomiting may include dehydration, sodium and potassium depletion, metabolic alkalosis, aspiration pneumonia, Mallory–Weiss syndrome, Boerhaave's syndrome, cachexia, post-surgical bleeding and post-surgical wound disruption.

Importantly, it was noted on numerous occasions by clinical investigators that after one or more courses of treatment for malignant tumours, patients might refuse to continue with potentially life-saving treatment because the side-effects of nausea and vomiting had become intolerable (6, 7). With increasing efficacy of therapeutic regimes for the control of malignancy, such side-effects looked like becoming the rate-limiting step in treatment. And yet at the meeting in Oxford in 1984, entitled *"Nausea and Vomiting: mechanisms and treatment"*, reviews of the success rate of drugs against vomiting from all causes showed that emesis remained relatively poorly amenable to pharmacological intervention.

Because of the sheer magnitude of the clinical problem, this book is biased toward consideration of the impact of advances in serotonin pharmacology on cytotoxic drug- and radiation-induced emesis. Nevertheless, it contains contributions from experts who have looked at other areas such as motion illness and postoperative vomiting. In practice, however, it was the advent of highly potent but essentially toxic chemotherapeutic drugs like cisplatin that gave renewed impetus to emesis research. Despite its frequency (even before the emergence of agents like cisplatin and techniques like whole-body irradiation), up until the 1960s treatment of vomiting was carried out largely on an empirical basis, using drugs such as phenothiazines and antihistamines, which had been shown to have an effect against gastrointestinal and vestibular-based vomiting. The first controlled trial against chemotherapy-induced nausea and vomiting was carried out in 1963 by Moertel and colleagues (8), comparing phenothiazines with placebo during the use of 5-fluorouracil, a mildly emetic drug. Before 1977, chemotherapy-induced nausea and vomiting received only scant attention with a mere total of six controlled therapeutic trials between 1963 and 1976 (9). With the appearance of novel cytotoxic agents and anti-emetics like the cannabinoids and domperidone, there was renewed interest in the area (6). There was a similar paucity of experimental work on emetic mechanisms, and apart from Borison's paper in 1958 on the site of action of mustine in the cat (10) it was not until the 1980s that attention was re-directed to this area of experimental physiology and pharmacology. The history of clinical studies into radioemesis is similar, but much more interest was shown in the mechanism of X-radiation-induced emesis, starting with Borison's work in the cat (11).

A SHORT HISTORY OF RESEARCH INTO THE PHENOMENON OF NAUSEA AND VOMITING

The early days

The first recorded experimental work on nausea and vomiting appears to be that of Wepfer in 1679 who observed the closure of the pylorus and contraction of the pyloric part of the stomach during vomiting in cats, dogs, and even wolves! (12). Later, in 1813, Magendie recounts in his "Memoire Sur Le Vomissement" an experiment in which a dog's stomach was replaced with a pig's bladder, after which vomiting could still be induced (13). It became accepted by the time that Magnus reviewed the literature in 1903 (14), and Hatcher again in 1924 (15), that there were at least two centres for vomiting, one of which was automatic, where apomorphine acted directly, and the other a reflex centre. Giannuzzi in 1865 (16) is credited with being the first worker to suggest the existence of a vomiting 'centre' *per se*, having made serious efforts to study the involvement of the CNS in antimony sulphate-induced vomiting in dogs. In the late 1880s Openchowski (17) and his pupil Hlasko (18) (quoted in (15)) carried out a great deal of work and contended that there were independent centres for various acts concerned with vomiting, with separate paths from each centre. Openchowksi's failure to induce emesis in dogs with intragastric copper sulphate after vagotomy also led him to the general conclusion that the vagi alone carried afferent emetic impulses from the stomach. It was Thumas in 1891 (19) who described a very small area (2 mm wide around the midline, extending from 2 mm anterior to calamus scriptorius posteriorly for 5 mm, through the obex) in the posterior part of the rhomboid fossa of the dog that was more sensitive than any other to the emetic apomorphine. When this area was destroyed, apomorphine failed to cause vomiting.

In 1898 Cannon described the contribution of the various muscle groups to the act of vomiting and defined the basic mechanics (12). These were later verified and expanded by Borison, Brizzee, McCarthy and others in the 1960s and 1970s (20–23).

Hatcher and Weiss confirmed and extended Thumas' studies with apomorphine in the mid-1920s and prevented vomiting induced with either apomorphine or mercuric chloride by destroying the ala cinerea (dorsal sensory nucleus of the vagus, i.e. the nucleus tractus solitarius) (24). Then in 1930 Koppanyi demonstrated in dogs with chronic lesions in the ala cinerea that the emetic sensitivity to parenteral apomorphine was reduced, whereas 'irritant' emetics (e.g. zinc or copper sulphate) remained effective by the oral route (25). The hypothesis that the vomiting centre was in the ala cinerea was thus placed in doubt. As late as 1948 various textbooks of neuroanatomy described several different neural structures as the vomiting centre, for example, the dorsal motor nucleus of the vagus, or the nucleus of Roller. All these centres, however, were postulated on the basis of a unitary concept, that both the central and reflex emetic agents elicit vomiting by activating the same neural structure, despite the evidence of Koppanyi (25).

The modern era

Central mechanisms

Before 1948, investigations by Miller and Sherrington (26) and others had not been successful in obtaining emesis by electrical stimulation of the medulla oblongata. Having decided that barbiturate anaesthesia caused a general CNS depression, this work was repeated by Borison and Wang in 1949 (27) in the decerebrate cat, and vomiting was apparently readily observed during these medullary stimulations, so ushering in a period of intense research and real progress in our understanding, associated with such names as Wang, Borison, Brizzee and McCarthy. In 1951, Wang and Borison appeared to seal their concept of the vomiting centre as localized in the bulbar reticular formation when they showed that its destruction resulted in a loss of the emetic response to both central and peripheral emetic stimuli (28). However, in 1983, Miller and Wilson (29) failed to duplicate Wang and Borison's 1949 electrical stimulation experiment and concluded that a discrete vomiting centre did not exist, feeling that the results of their experiments were more consistent with the concept that neurones involved in the control of vomiting are diffusely distributed to the effective region described in 1949. They suggested that electrical stimulation may be successful in eliciting emesis by direct excitation of descending pathways, which could produce coordinated activation of the vomiting musculature (30).

To address this conflict, an alternative organizational model of this central control system was proposed by Davis, Leslie, Harding and Andrews in 1984, based on the idea of sequential activation of the various effector nuclei comprising the vomiting act. In contrast to the conventional view, the vomiting centre was envisaged not as a discrete entity but a higher and integrated function of these separate effector nuclei (Figure 1) (31). Subsequently it has been speculated that a set of more or less diffuse neurones acting to coordinate the vomiting act (the so called 'central pattern generator for emesis') may be located in the nucleus tractus solitarius (32). This proposal is particularly interesting in a historical context as Hatcher and Weiss had already commented back in 1923 (24) that "It therefore seemed logical to look to the sensory nucleus of the vagus for the seat of the vomiting reflex".

After their work on the vomiting centre, Borison, Wang and colleagues proceeded to carry out superficial lesions of the caudal brainstem in the region of the ala cinerea in dogs, which resulted in abolition of the vomiting response to apomorphine and certain glycosides given intravenously. By 1953 they published their now classic hypothesis that the chemoreceptor trigger zone, as the region had become known, has a receptive not an integrative function and that it communicates with the vomiting centre (3). Interestingly, this issue of the extent to which any integration occurs within the area postrema is still a matter of debate today. Subsequently, Borison demonstrated the functional distinction between the chemoreceptor trigger zone and the vomiting centre (33). Borison and Wang (3) defined the chemoreceptor trigger zone in the dog as a bilateral structure less than 1 mm^3 in size, on either side of the

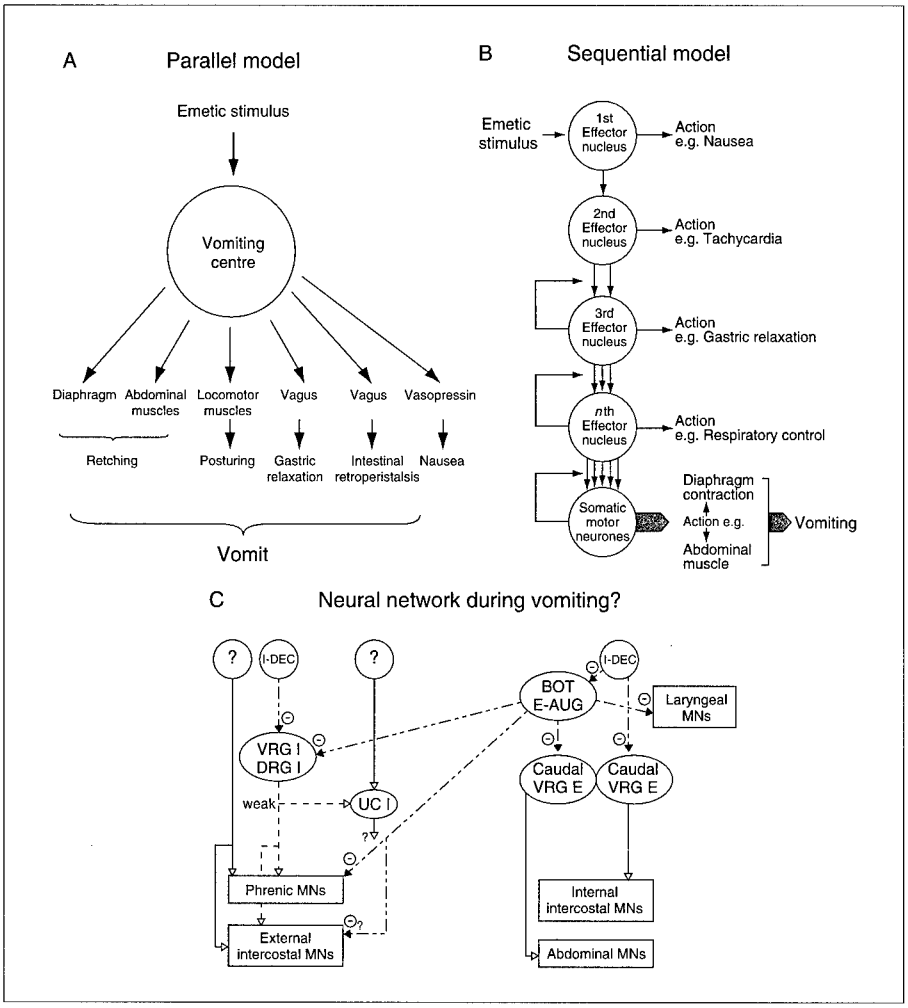


Figure 1. Different types of model of the 'vomiting centre' from the black-box conceptual approaches of parallel and sequential models (A and B) of Davis *et al.*, reproduced with permission from (31), to the neural network model of Miller (C), reproduced with permission from AD Miller. I-DEC, decrementing inspiratory; VRG I, ventral respiratory group; DRG I, dorsal respiratory group; UC I, upper cervical; BOT, Bötzinger; E-AUG, augmenting expiratory; MN, motoneurons.

fourth ventricle, contiguous medially with the area postrema. Based upon their experimental studies, they produced a diagram of the organization of the emetic reflex (Figure 2). As with the diagrams of the vomiting centre, this diagram has had an enormous influence on our concept of the reflex and to some extent all the more

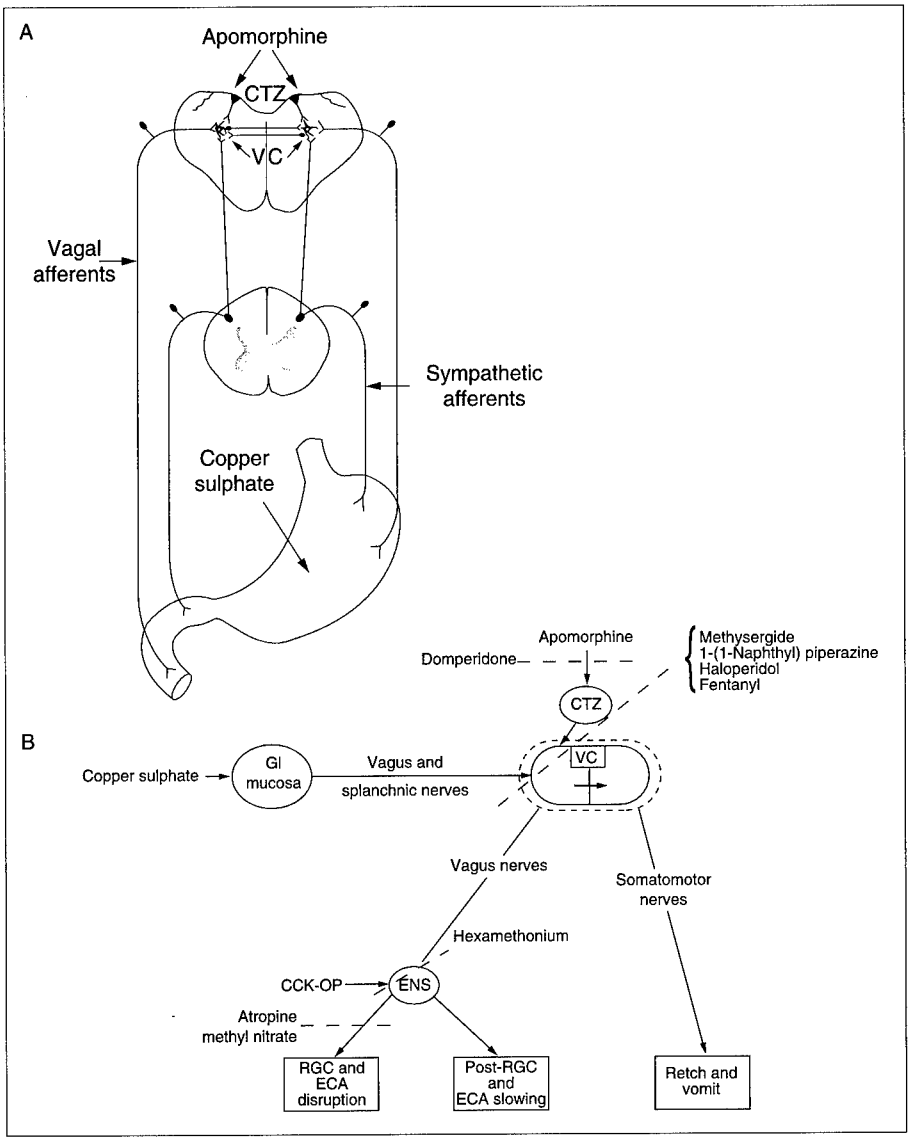
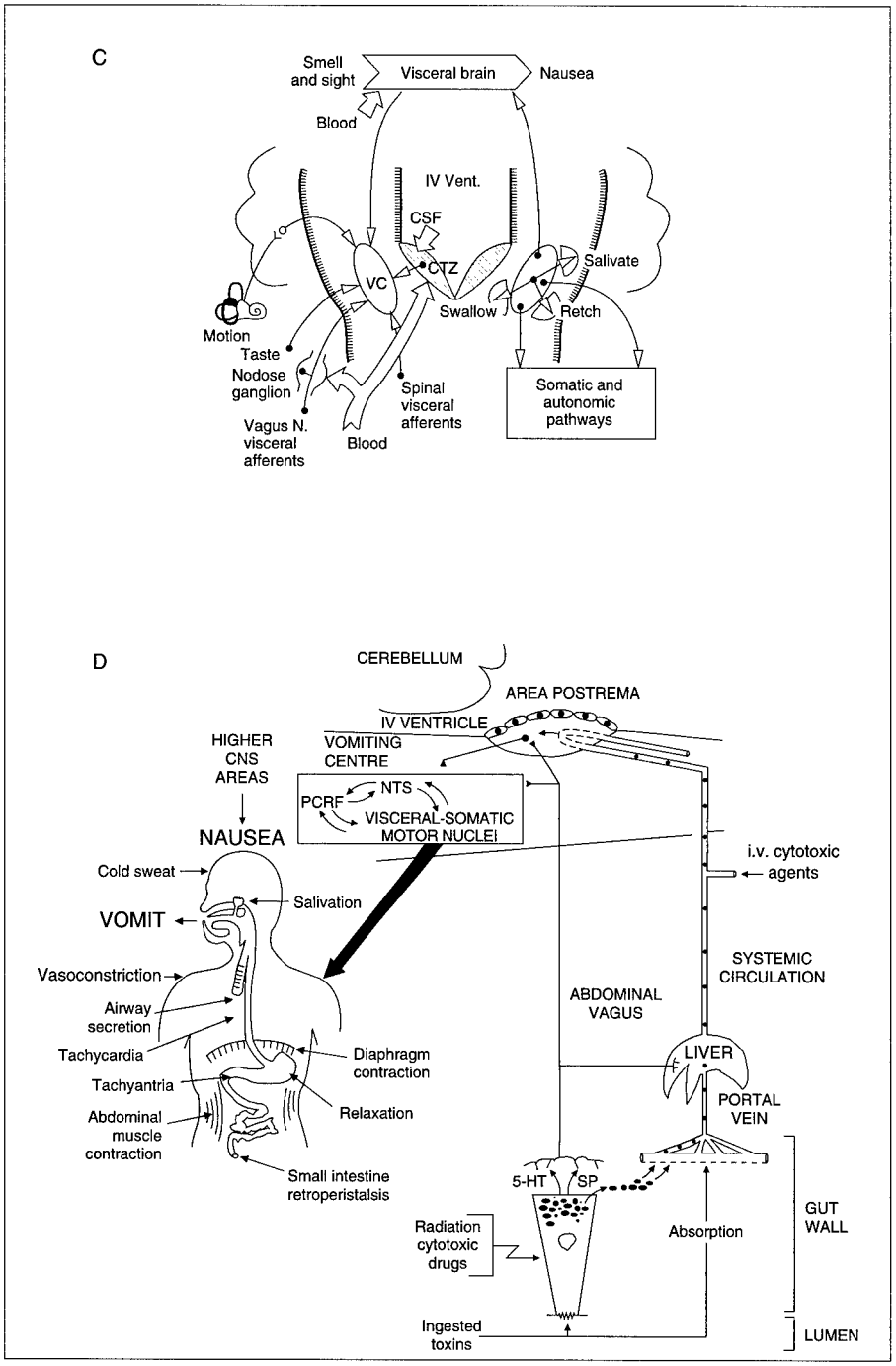


Figure 2. Different types of schematic representation of the organization of the emetic reflex from the original Wang and Borison (A) 1952 model (66), to Lang and Marvig (B) in 1989 (70), Borison and McCarthy (C) in 1981 (69), and Andrews, Rapeport and Sanger (D) in 1988 (67). Note that all the models contain essentially the same key elements. CTZ, chemoreceptor trigger zone; VC, vomiting centre; CCK-OP, cholecystokinin octapeptide; ENS, enteric nervous system; RGC, retrograde giant contraction; ECA, electrical control activity; NTS, nucleus tractus solitarius; PCRF, parvicellular reticular formation; 5-HT, 5-hydroxytryptamine; SP, substance P. Figures 2A and 2D are reproduced with permission from (66) and (67) respectively.


C
Smell and sight
Visceral brain
Nausea
Blood
IV Vent.
CSF
CTZ
VC
Salivate
Swallow
Retch
Motion
Taste
Nodose ganglion
Vagus N. visceral afferents
Blood
Spinal visceral afferents
Somatic and autonomic pathways
D
CEREBELLUM
AREA POSTREMA
IV VENTRICLE
HIGHER CNS AREAS
VOMITING CENTRE
PCRF
NTS
VISCERAL-SOMATIC MOTOR NUCLEI
NAUSEA
i.v. cytotoxic agents
Cold sweat
Salivation
VOMIT
Vasoconstriction
Airway secretion
Tachycardia
Tachyantria
Abdominal muscle contraction
Diaphragm contraction
Relaxation
Small intestine retroperistalsis
SYSTEMIC CIRCULATION
ABDOMINAL VAGUS
LIVER
PORTAL VEIN
5-HT
SP
Radiation cytotoxic drugs
Absorption
GUT WALL
Ingested toxins
LUMEN

recent organizational diagrams contain the same elements (see Figure 2). Such organizational diagrams are also of importance as they provide a conceptual framework within which sites of action of emetics and anti-emetics are discussed.

The elucidation of the neuronal connectivity of the area postrema began with the recognition of fibre connections between it and the subjacent nucleus tractus solitarius (34–36). Physiological evidence for a neuronal connection from the area postrema to the vomiting centre was obtained by Iwase *et al.* (37), who elicited vomiting-like behaviour in the dog by electrical stimulation of the chemoreceptor trigger zone, which earlier studies by Borison and Wang, and Miller and Wilson, were not able to detect. By 1985, in his review of the topic (38), Leslie was able to say that noradrenaline, adrenaline, dopamine, serotonin and GABA had been localized in the area postrema of the cow, consistent with work in other species (39). Other studies provided evidence (usually immunohistochemical) for the presence of cholecystokinin, neurotensin, angiotensin II, prolactin and insulin, or their binding sites in the area postrema of a variety of animals (e.g. rats, other rodents and primates) (38). A number of neurotransmitters and peptides were administered to animals with the idea of characterizing the responsiveness of neurones of the area postrema and by 1983, Brooks and colleagues had attempted recordings in an isolated brain tissue preparation and found neurones unresponsive to glutamate, serotonin, angiotensin II, dopamine and osmotic changes (40). However, Carpenter (41, 42) carried out extensive work recording from area postrema neurones in the dog, in which 17 common transmitters and peptides were employed. Excitatory responses were found to glutamate, histamine, noradrenaline, serotonin, dopamine, apomorphine, angiotension II, neurotensin, leu-enkephalin, vasoactive intestinal protein (VIP), thyrotrophin-releasing hormone (TRH), gastrin, vasopressin and substance P. It is still not clear how these results relate to the emetic mechanisms activated by cytotoxic drugs and radiation, but they do support Leslie's observations of the area postrema and related structures as a region of striking neurochemical complexity.

Interestingly, there is some direct evidence for the role of the area postrema and chemoreceptor trigger zone in man. This work was carried out in 1962 in five patients suffering from untreatable vomiting resulting from inoperable brain tumours. At craniotomy, the topography of the calamus scriptorius was found to be very similar to that of the dog, cat and monkey and, in each case, the area postrema was lesioned, with the result that vomiting was relieved, and moreover, the patients also became refractory to apomorphine when challenged after complete recovery from the surgery (43).

An important advance in our understanding of the central organization of emetic mechanisms came from the studies of Costello and Borison in 1977. They found that the anti-emetic effects of opioids were blocked by naloxone given systemically, but that the emetic effects were only blocked by naloxone given intracerebroventricularly (44). They suggested the existence of an 'anti-emetic centre' in the reticular formation, which exerted an endogenous anti-emetic force mediated by enkephalins. The significance of this work may have been underestimated because, if the endogenous inhibitory mechanisms were fully understood, they could provide

a novel pharmacological approach to anti-emetic therapy (see also studies by Zabara *et al.* below). Enkephalins were included in a major hypothesis put forward by Harris in 1982 concerning the mechanism of cytotoxic drug-induced vomiting, which involved the effects of enkephalins on specific opioid receptor subtypes distributed between such emetic and anti-emetic centres (45). It was proposed that cytotoxic drugs might act by inhibiting different phases of the synthesis of enzymes involved in the breakdown of enkephalins, leading to an increase in their concentration at a critical point in the emetic pathway, which in turn would lead to an activation of emesis. This hypothesis was one of the first to attempt to provide a unified mechanism for the way in which a chemically diverse range of cytotoxic drugs activates emesis. The search for a unified mechanism continues, with attention now focused on the generation of free radicals in enterochromaffin cells (see Chapters 5 and 6).

Peripheral mechanisms

Around 1910, Miller studied the afferent nerves to the 'vomiting centre' using the emetic action of mustard seeds, and concluded that the vagi alone were responsible for this function, as immediately following vagotomy, emesis was no longer capable of being elicited, which was not the case after section of the splanchnic nerves (46). The importance of afferents in the vagus was further stressed by Bayliss in 1940 who produced vomiting by intraperitoneal injection of staphylococcal enterotoxin, and found that emesis rarely occurred following division of the vagi (47). Interestingly, irritation or distension of the small intestine was more effective in inducing emesis than similar stimulation of the stomach, as Keeton had found in 1925 (48). Generally, the abdominal vagal afferents were found to be more important than afferents travelling in the splanchnic nerves. The importance of the vagal afferents was recognized as early as 1910 by Miller, who elicited vomiting readily through electrical stimulation of the cut central ends of the vagal branches supplying the stomach.

In 1972 Zabara and colleagues introduced the concept of neuroinhibition in the regulation of emesis (49). Emesis preceded by retching could be induced in the dog by electrical stimulation of the abdominal vagal nerves at the supradiaphragmatic level. So, Zabara suggested that failure to produce retching or emesis by similar stimulation of the cervical vagal trunk meant that, either the abdominal vagal afferents did not travel in the cervical vagus, or that inhibitory fibres were present. They concluded that inhibitory fibres were present, as the retching and vomiting resulting from stimulation of the supradiaphragmatic vagus could be prevented either by transection of the cervical vagus or by simultaneous stimulation of the cervical vagal trunk. They maintained that the excitatory system, involving the 'vomiting centre' and chemoreceptor trigger zone, acts in conjunction with an inhibitory system so that emesis is normally prevented by a dominance of inhibition over excitation.

At the centre of the vomiting reflex lies the gastrointestinal tract and although vomiting continues in its absence, the gut remains the organ around which the reflex,

Table 1. The hierarchical organization of the defence mechanism for protection of the organism against toxins.

Level of defence mechanism	Location of toxin	Type of sensor and location	Effect	Result
First	External to gastrointestinal tract	1. Smell and taste 2. Peripheral	(a) Avoidance (b) Nausea	(a) Learned aversion for potential toxins
Second	Intragastric	1. Gastric chemo-receptors 2. Near the absorptive site	(a) Nausea (b) Gastric motility ↓ (c) Avoidance (d) Vomiting	(a) Learned aversion (b) Confining toxin to one area (c) Ejection of toxin
Third	Within the vascular system	1. Area postrema (CTZ) 2. Within the CNS	(a) Nausea (b) Gastric motility ↓ (c) Vomiting	(a) Ejection of toxin (b) Confining toxin (c) Learned aversion

in its present form, has developed. Drawing on the ideas of the previous authors (e.g. Hatcher and Weiss (24)), and a review of the concepts and data presented at the 1984 Oxford meeting on emesis (50), Davis, Harding, Leslie and Andrews proposed the view that the defence of the organism against toxins is organized into a tiered or layered system (Table 1, (31)). This hierarchy of defensive measures is designed to protect the organism against increasing penetration by toxins of various types. It was proposed that viewing the system in this way would go some way to explaining the disparate responses of different species to similar stimuli, and perhaps similar responses of individuals to different stimuli. For instance, it is generally accepted that laboratory rats do not vomit; they do, however, develop a profound conditioned taste aversion to wide-ranging stimuli, including X-radiation and toxins, which cause vomiting in other species. The omnivorous rat displays a bias, probably established by natural selection, to associate gustatory and olfactory cues with internal malaise even when these stimuli are separated by long time periods. The first line of defence in the rat (i.e. smell, taste) appears to be extremely highly developed and, therefore, perhaps as a result, the third and last line has become redundant and is now non-functional. In laboratory rats even the second line of defence (located in the gut) seems to be obsolescent because although it is known that gastric afferents in the rat respond to intragastric copper sulphate, vomiting does not result. The detection apparatus is present, but the vomiting response absent. Essentially, here we have an example where the primary defence mechanism is so highly tuned that the animal no longer needs to rely on the other two lines of defence downstream, so that in this animal the latter are no longer effective. The 'reality check' for us in 1984 when we put together this idea was to look for other physiological systems to which it might apply. In the case of the mammal there are only two systems 'designed' to absorb into the body exogenous substances from the environment. Both systems are fundamental to sustaining life and, therefore, we

Table 2. A hierarchical organization of the respiratory defence mechanism.

Level of defence	Sensor and location	Protective mechanism
First	Nasopharynx and upper airways receptors	Sneezing and coughing reflexes
Second	Lungs; various pulmonary receptors	Hering-Breuer and lung irritant reflexes
Third	Carotid, aortic and medullary chemoreceptors	Respiratory chemoreceptor reflexes

would predict, require adequate protection. The other comparably organized system is the respiratory system (Table 2).

The mucosa of the gastrointestinal tract plays a particularly important part because although it represents the second layer of defence, it is the last barrier to toxic molecules before they enter the body of the organism proper (51). The gut, then, functions as both the absorptive site for food and a sensor mechanism for recognition of potentially toxic material. Up to 1984, researchers had for some time concentrated their efforts on investigation of the effects of area postrema ablation, as emphasis had appeared to shift from the periphery to the CNS after the delineation of the chemoreceptor trigger zone within the area postrema and its distinction from the 'vomiting centre'. Few papers had been published (exceptionally, for instance, 49, 52–54) that examined the effect of abdominal vagal section on vomiting, since the series by Borison and his co-workers in the 1950s (55, 56). Concentration on the area postrema and the effect of its destruction on various types of vomiting, however, produced yet more controversy because the procedure is a technically difficult one and brings with it no guarantee that the animal will even survive the surgery on the brainstem because of potential damage to neuronal systems controlling respiration, for instance.

Animal models of man: a continuing debate

Before I draw this review of the last 300 years to a close, there is one more thing to mention – animal models. For the century up to 1980, three animal models dominated research into nausea and vomiting, namely, the dog, the cat and the primate. Studies confining themselves to elucidating the structure and function of the area postrema had been carried out largely in the rat, as had those on conditioned taste aversion. However, Brizzee and coworkers had already noted in 1955 "that the chemoreceptor trigger zone for emesis in the monkey is virtually non-functional from the standpoint of drug action" (57) and the ED_{50} for radio-emesis is twice that in humans, at about 450 cGy. The cat too has a very high threshold to radiation-induced emesis, of the order 2–5000 cGy. The dog had, until the 1980s, been the animal model of choice for over 170 years and its emetic responses are closely analogous to humans. But all three animals were becoming very expensive,

increasingly difficult to obtain, and moral and ethical objections were being raised ever more strongly against their use. With the renewed impetus for research in this area, fuelled by the requirement to solve the cancer therapy side-effects problem, there was a need for an alternative animal model. The ferret – *Mustela putorius furo* L. was the animal that emerged as that new model, largely because it is a small, inexpensive carnivore that vomits to apomorphine. Two reports in 1981 and 1982 by Florczyk, Schurig and Bradner raised the profile of the ferret, with successful experiments on cisplatin-induced vomiting (58, 59), and it was they who proposed using the ferret for future work in this area. Coincidentally, Andrews and Scratcherd had already published extensively on the physiology of the reflex vagal control of the ferret stomach and had elicited retching to vagal afferent stimulation under urethane anaesthesia (60). At the time I started work in Oxford in 1983, the ferret was the natural choice as the experimental animal. However, its response to a wide range of emetic agents was poorly characterized and required lengthy initial studies to determine whether it could provide an appropriate and reliable model for studying the mechanisms of cytotoxic drug- and X-radiation-induced vomiting in humans. A variety of pharmacological and surgical techniques were used, focusing particularly on the role of the abdominal innervation. In addition, the 2-deoxyglucose autoradiography technique was used to study the brainstem pathways involved in emesis (61). This was the first use of such a brain-imaging technique to study emesis in the ferret. In parallel, Sanger and Miner began to use the ferret for their explorations of serotonin pharmacology and emesis, followed by Costall, Domeney, Naylor, Gunning and Tattersall, the early results of these studies being published in 1986 (62, 63).

The issue of the most appropriate animal model for emesis in humans is still the subject of discussion not only from an academic viewpoint but also because of the importance of predicting the outcome of clinical efficacy from preclinical studies. In the past 5 years, two new models have emerged, the pig (64; see Chapter 19), and an insectivore, the house musk shrew, *Suncus murinus* (see Chapter 6). In addition, by modification of dosing protocols a model for delayed emesis has been developed in the ferret (see Chapter 22). The predictive value of these newer models is not yet known but whatever the outcome, all the studies serve to increase our overall understanding of emesis, which can only advance the design of better anti-emetic treatment.

EPILOGUE OR PROLOGUE?

And so we reach the 1984–5 time-frame and the last Oxford meeting (50). At that point however, research had reached an impasse. What was needed was a pharmacological tool that would match our physiological techniques for elucidating the pathways involved in control of cytotoxic- and radiation-induced nausea and vomiting. There were few, if any, clues in 1984 as to what that might

be, for serotonin was mentioned on precious few occasions. Yet within 2 years, Miner and Sanger had published their seminal paper on the prevention of cisplatin-induced emesis in the ferret using a 5-HT_3 receptor antagonist (62) and within 10 years the sales of such drugs had reached many millions of pounds sterling worldwide. For all of those who had been involved in studying emesis for a number of years, the world changed from an emphasis on studies involving the area postrema and its role, to exploring our observations that vagotomy paralleled the effect that the 5-HT_3 receptor antagonists had on emesis (65). Wang and Borison's 1952 'wiring diagram' (66) evolved into something altogether more satisfying (Figure 2D) (67).

For the patient, the most important element in all this endeavour, this breakthrough – for it can justifiably be called that – has meant a significant increase in the quality of life and I am sure in some cases it is no exaggeration to say, the chance of life itself.

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Chapter 2

The physiology of emesis induced by anti-cancer therapy

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INTRODUCTION

Cytotoxic drugs and radiation, both of which are used alone and in combination in the treatment of cancer, are potent inducers of nausea and vomiting in humans and a variety of other mammals (e.g. cat, dog, monkey, ferret and house musk shrew *Suncus murinus*; see below for references). The emetic effects of cytotoxic drugs such as emetine (an inhibitor of protein synthesis originally used for the treatment of amoebiasis) have been known for centuries, as it is one of the ingredients of the clinical emetic, syrup of ipecacuanha, but in the modern era the emetic effects of nitrogen mustard gained prominence during the First World War (1). The first published descriptions that characterized the emetic effects of radiation date from the late 1940s and early 1950s with the studies of Zugerman (2) and Court-Brown (3). The similarities between the effects of radiation and cytotoxic drugs were noted in some of the earliest studies and nitrogen mustard in particular is often described as 'radiomimetic'.

The latency, magnitude and pattern of the emetic response to cytotoxic drugs depends to a large extent on the agent itself, but cisplatin (used in the sucessful treatment of solid tumours, e.g. ovary, testis) is often used in experimental studies, as clinically it is considered to be amongst the most emetic of agents. Some drugs such as vincristine and bleomycin are weakly emetic, and hence, are rarely studied. The reason for these differences is far from clear; earlier studies suggested that the emetic potential was related to protein synthesis inhibition (4), but in the light of more recent studies perhaps we should investigate their ability to generate free radicals (see Chapter 6), or to release 5-hydroxytryptamine from enterochromaffin cells (see Chapters 5 and 7). Like cytotoxic drugs, the emetic response to ionizing radiation is also dependent on the type and dose, but in addition, the site of exposure is also important, with the upper abdomen being the most sensitive (see Chapter 12).

The mechanisms and pathways by which these agents induce acute emesis have been the subject of direct experimental study for many years (see Chapter 1) with the modern era probably beginning with the experiments of Hatcher and Weiss in the early 1920s, and continued by the extensive investigations by Wang, Borison, McCarthy and Brizzee beginning in the late 1940s. These workers, and in particular Wang and Borison, laid the foundation for current concepts of the physiology of emesis. The basis for this Chapter is to reassess some aspects of the earlier and more

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recent work in the light of contemporary studies, and in particular, the insights into mechanisms and pathways provided by the 5-hydroxytryptamine-3 ($5\text{-}HT_3$) receptor antagonists over the past decade.

THE MECHANICS OF EMESIS

The emetic response is conventionally considered to encompass the subjective sensation of nausea (derived from the Greek word for ship) in addition to the expulsive mechanical components of retching and vomiting. In the main, this chapter will confine itself to retching and vomiting because the physiological mechanisms that underlie the genesis of the sensation of nausea are far from clear (see abstract P9, and Chapters 5 and 6).

Three phases of emesis are commonly described in response to cytotoxic drugs, particularly cisplatin; these are anticipatory, acute and delayed emesis (Figure 1). Whichever the phase of the response (i.e. time of occurrence in relation to cytotoxic drug administration), the mechanical aspects are the same, consisting of episodes of retching and vomiting, and each is assumed to have the associated visceral prodromal components (e.g. gastric relaxation, retrograde giant contraction of the

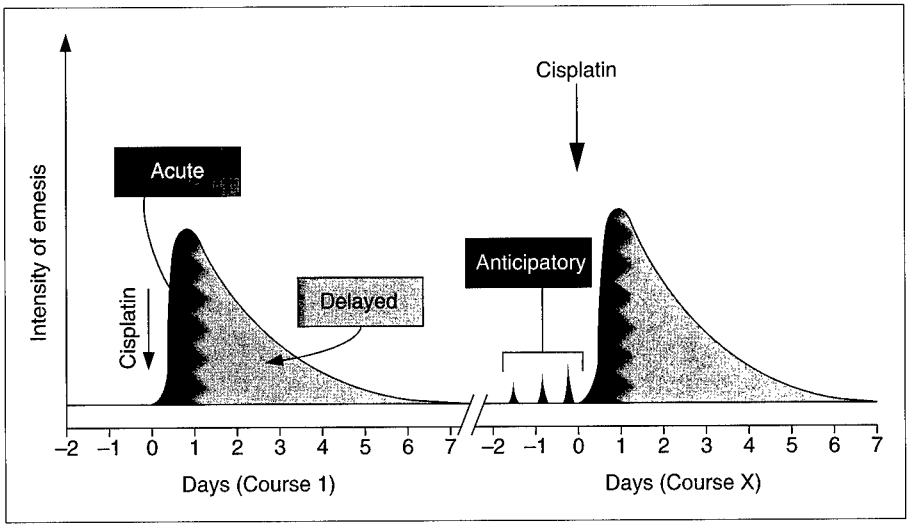

Figure 1. The types and pattern of emesis associated with high-dose cisplatin administration in humans. The acute phase (sensitive to $5\text{-}HT_3$ receptor antagonists) is conventionally considered to last 18–24 hours, and is followed in some patients by a protracted but less intense delayed phase (see Chapter 19). Although some patients may experience anticipatory nausea and vomiting before the first course of therapy it is more commonly encountered on the second and subsequent courses. A psychological component may be involved in the delayed phase but it does not appear to explain the bulk of the response.

small intestine) although this is not known from experiments. The mechanics of retching and vomiting are shown in Figure 2, and for more detailed accounts the reader is referred to Lang (7) and Monges *et al.* (8).

The mechanical components of retching and vomiting are mediated by both the autonomic and somatic divisions of the nervous system (comparable to the cough reflex) with the vagus playing the predominant role in the changes in gut activity, and the phrenic nerve regulating the diaphragm. The activity of these and other motor outputs is coordinated in the brainstem. Although the term 'vomiting centre' provides a useful shorthand for the anatomical substrate of the brainstem nuclei involved in coordinating the emetic response, the term 'emetic pattern generator' probably conveys a more accurate impression of current thinking. Because the somatomotor component of retching and vomiting involves the respiratory muscles, the search for the 'vomiting centre' has focused on the brainstem nuclei involved in the generation of the breathing rhythm (9–13): that is, the dorsal respiratory group located in the ventrolateral subnucleus of the nucleus tractus solitarius, ventral respiratory group comprising neurones located in the region of the nucleus ambiguus, including the nucleus retroambigualis and the Bötzinger complex. Some studies have suggested that this latter nucleus is the central emetic pattern generator, although this conclusion has been challenged (11) on technical grounds and because only the coordination of the respiratory components of emesis were investigated. By

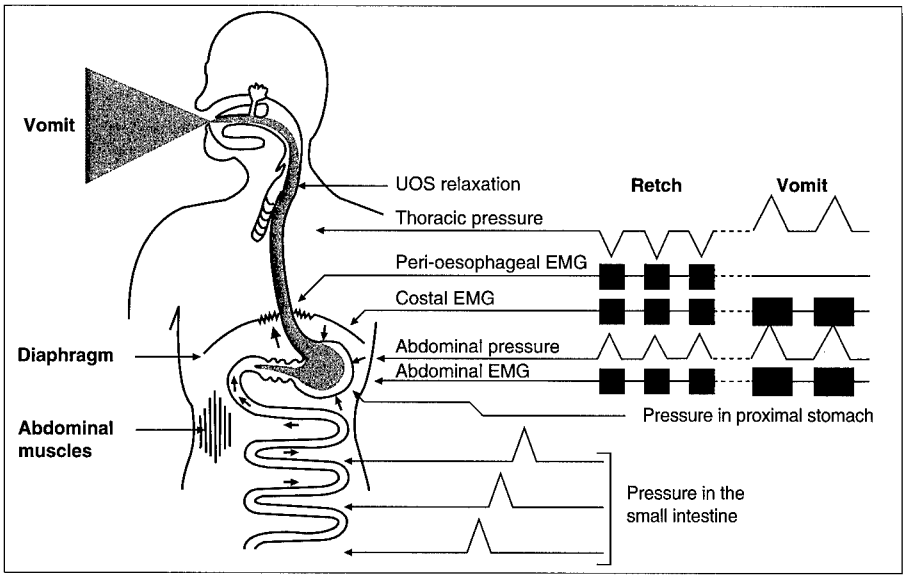

Figure 2. The major mechano-motor components of retching and vomiting. EMG, electromyogram; UOS, upper oesophageal sphincter. For details, see text and (7) and (8). Reproduced with permission from (4).

its very nature, if there is a single pattern generator it should regulate the visceral (mostly prodromal), as well as the somatic, components of emesis. It is, of course, conceivable that there are separate visceral and somatic pattern generators that interact sequentially, and such a system was suggested on theoretical grounds by Davis *et al.* (14) and is supported to some extent by the experimental studies of Lang *et al.* (7), and De Ponti *et al.* (15). The organization of the reflex is clearly very complex, especially when it is considered that although nausea, the gastrointestinal prodromal motor components, retching and vomiting usually occur in a temporal sequence, experimentally they can be separated (7). In investigating potential anti-emetic drugs it is important that they block all components of the emetic response and not only the overt somatic components of retching and vomiting. It is worrying that we still do not know the effect that 5-HT_3 receptor antagonists have on the gastrointestinal components of emesis in either animals or patients given cytotoxic drugs (see P7, P8). The less than complete efficacy of 5-HT_3 receptor antagonists in humans could be influenced by a failure to block completely the gastrointestinal components of emesis. For example, if the retrograde giant contraction is still present, bile reflux into the stomach will irritate or sensitize the gastric mucosa. This, together with disordered gastrointestinal motility, could generate abnormal visceral afferent activity, inducing nausea and vomiting.

IS EMESIS AN APPROPRIATE RESPONSE TO ANTI-CANCER THERAPY?

Although animals try to avoid ingesting potentially hazardous material by using the senses of vision, taste and smell, accidental ingestion may occur when animals bolt food with little if any prior tasting (e.g. sharks, some carnivorous mammals). In these cases the toxin may be disguised, or in the case of bacteria may not be present in sufficient quantities until the bacteria multiplies in the host's gut. Vomiting is a widespread protective reflex (it is present even in some invertebrates e.g. pleurobranch molluscs (16)), which serves to expel in bulk, accidentally ingested toxins from the upper gastrointestinal tract. The sensation of nausea serves as a warning (comparable to pain), and usually results in the cessation of ingestion (as with limb withdrawal from a noxious stimulus) but in addition, arguably its main function is in the generation of an aversive response to the toxin or the food in which it was contained so that it will be avoided if encountered again. Teleologically, it can be proposed that vomiting should not be aversive, as it serves to expel the toxin from the body, and anecdotal reports that immediately following vomiting a sensation of well-being is often experienced, suggest that the body may have a mechanism for reinforcing this behaviour, possibly involving the release of endorphin. This hypothesis requires experimental investigation.

The above responses are all appropriate in the context of the natural world. In a clinical context when patients receive anti-cancer therapy, activation of this system

that evolved to respond to ingested toxins occurs, but it is inappropriate because:

1) the vomiting does not remove the perceived toxin from the circulation. Therefore, in contrast to when the stimulus is in the gut lumen, vomiting is not self-limiting and this probably contributes to its protracted nature. In addition, vomiting further burdens the patient with fluid and ionic loss, physical trauma and nutritional problems.

2) The nausea generates an aversion and hence avoidance of the stimulus (i.e. the anti-cancer therapy). This may lead to failure to attend for regular cycles of therapy, possibly leading to an increase in morbidity and mortality.

3) The patient may develop anticipatory nausea and vomiting which is very difficult to treat once established and which, if it continues during therapy may add to the acute and delayed emesis induced directly by the anti-cancer therapy. This could reduce the efficacy of 5-HT_3 receptor antagonists in the acute phase.

In view of this cascade of effects it is clear that the best approach is to intervene early in the process before nausea and vomiting begin (Figure 3). It is only by understanding the mechanisms and pathways by which anti-cancer therapies induce emesis that we will be able to make rational decisions about the most effective site at which to target pharmacological treatment.

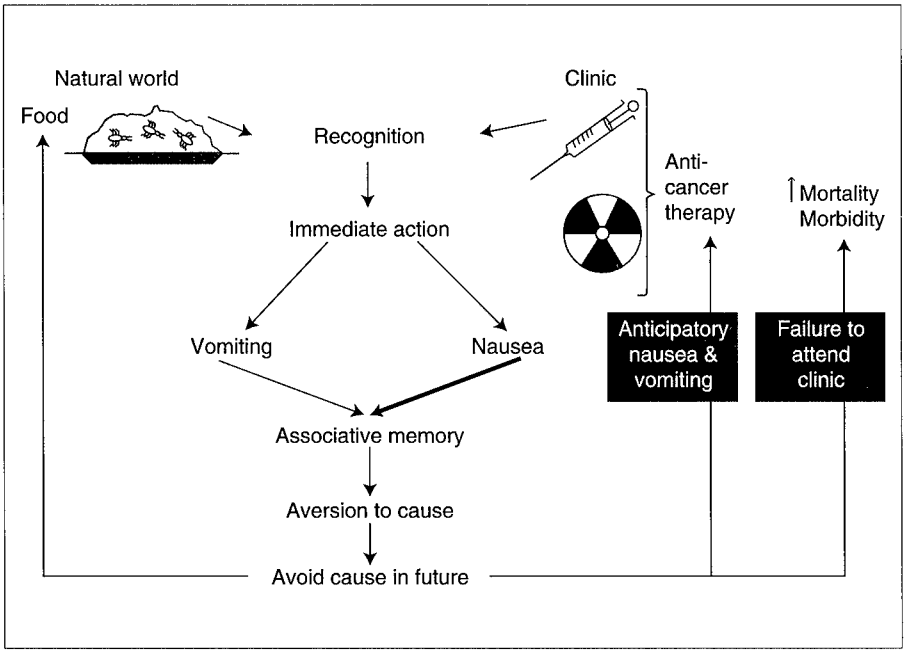

Figure 3. The key steps in the nausea and vomiting response to an ingested toxin in contaminated food compared with the response to cytotoxic therapy. The bold arrow indicates the more pronounced effect that nausea has in generating an associative memory.

PATHWAYS INVOLVED IN THE INITIATION OF THE EMETIC RESPONSE TO ANTI-CANCER THERAPIES

This section, which forms the major part of the review, is divided into two parts; the first dealing extensively with the general problems of interpretation of the experimental studies undertaken to provide a background to the second part, the discussion of the studies themselves.

Problems of interpretation

Although the aim of many of the studies of the pathways involved in the response to anti-cancer therapies is to aid in the targeting of drug therapies, it is impossible to undertake the appropriate studies in man. There is considerable interest, therefore, in identifying which experimental animal species is closest to humans in its emetic response. Irrespective of the species used, it must be borne in mind that it is the emetic response to a particular agent that is under investigation and not the response *per se* to anti-cancer therapy. Unlike human patients, the animals do not have a tumour load, are not hydrated (but see Chapter 19), do not have concomitant medication or surgery, and as far as can be ascertained are not subject to the same psychological factors. In addition, the animals represent a less variable population in terms of genetics, age and previous emetic history (e.g. motion, pregnancy, food poisoning). Therefore, whatever mechanisms (particularly neurochemical) and pathways are identified, the results may not translate precisely to humans beacuse of these additional factors. In addition, because it is impossible to duplicate the surgical studies in humans and certainly not in healthy volunteers, we may never know for certain what pathways there are in humans. It is worth recalling that the only direct evidence that the area postrema is involved in emesis in humans is from the five patients in whom the effects of apomorphine were studied before and after area postrema ablation (17). There is probably even less direct evidence for an involvement of the abdominal vagal afferents, although stimulation of the central end of the vagus is known to produce nausea, but not pain in humans (18). Bearing these issues in mind we can now discuss the animal evidence for the pathways involved in the emesis induced by anti-cancer therapies.

This area has been fraught with scientific controversy because of the technical issues associated with the lesions, their interpretation, and the possible existence of species differences in the contribution of the various pathways. The problem has been compounded because of some persistent, but incorrect, views of the role of the area postrema. The latter was of concern to Borison and McCarthy who wrote in 1983 "we detect a spreading impression that the CTZ is in effect the universal chemosensory organ for emesis. Not only is this notion incorrect with respect to classical emetic pharmacology but its extension to chemotherapy-induced vomiting is bound to be misleading without specific proof of action on the CTZ" (19).

We can now begin to ask what experimental evidence would constitute such proof, and discuss the technical limitations of the various types of study before

discussing the results that have been obtained and providing a hypothesis that goes some way to reconciling some of the apparently discordant literature.

Area postrema ablation

This is a technically difficult procedure whatever physical method is chosen for the lesion (e.g. diathermy) and it is carried out under direct visual control rather than stereotactically, with the approach usually being to 'sculpt' the area postrema from the walls of the caudal fourth ventricle. The extent of the lesion is based upon experience of survival, recovery, functional assays, and histology. The major problem is that in all species, immediately subjacent to the area postrema is the nucleus tractus solitarius. This raises several anatomical problems of interpretation.

1) The subnucleus gelatinosus of the nucleus tractus solitarius is the major site of termination of abdominal vagal afferents. Earlier workers were well aware of this problem, as the following quotation from Brizzee *et al.* (20) in 1955 shows:

> This raises the question as to whether the trigger zone lesions (centering on the area postrema) may result in a loss of the emetic respone to X-irradiation through incidental damage to the dorsal sensory vagal nucleus... damage might conceivably interrupt an afferent pathway from visceral receptors of the vagus nerve to the reticular vomiting centre.

Brizzee *et al.* (20) were also equally cautious in their interpretation of their area postrema ablation studies:

> Results observed in the present study corroborate our findings in a previous investigation with monkeys, the weight of evidence favouring area postrema as the central site of mediation of X-irradiation emesis or the locus of an afferent pathway concerned with this reflex.

If the site of action of an emetic agent is investigated solely using area postrema ablation then even if the response is abolished it cannot be concluded with certainty that the area postrema was the site of action. Irrespective of how good the histological demarcation, the area of functional collateral damage cannot be accurately assessed. Although attention has focused on the vagal terminations, more generalized damage to the nucleus tractus solitarius could interfere with its proposed more general integrative role.

2) In several species, abdominal vagal afferents have been described that terminate in the area postrema itself (21). The function of this pathway is not known but it could provide a means of comparing the levels of chemicals in the gut lumen or peripheral circulation with those in the cerebrospinal fluid, or cerebral vasculature. If this relay pathway was involved in emesis then lesion of the area

postrema would abolish emesis even though the primary site of emetic action was in the gut and involved activation of vagal afferents. Studies investigating the effect of area postrema ablation in the ferret have identified animals in which the emetic response to loperamide (a centrally acting opioid receptor agonist), intragastric copper sulphate, and intraperitoneal cisplatin were blocked by area postrema ablation (Watson, Kovacs and Andrews, unpublished observations). Previous studies in the ferret have shown that, as in other species (e.g. dog, monkey, *Suncus murinus*), the emetic response to copper sulphate and cisplatin can be markedly reduced or abolished by abdominal vagotomy in combination with greater splanchnic nerve section (see below for discussion of cat data). The observation that the responses to these agents in the ferret can also be abolished by area postrema ablation suggests that in the ferret the abdominal visceral afferents either relay via the area postrema or pass sufficiently close in the subjacent nucleus tractus solitarius to be included in the lesion. In these lesioned animals it was possible to demonstrate that electrical stimulation of the abdominal vagi (under urethane anaesthesia) was capable of inducing an emetic response consisting of licking and mouth opening followed by a burst of retching. How can these two observations be reconciled? There are several possibilities, but one of the simplest is that the vagal afferent pathway mediating the response to copper sulphate and cisplatin (both involving the gut mucosa chemoreceptors) projects to the area postrema (a chemoreceptor) or to the subjacent subnucleus gelatinosus dendrites, which are likely to be damaged when the area postrema is lesioned, whereas another population of abdominal vagal afferents takes a different intra-medullary routing such that they escape damage. This population could be the afferents arising from the other major functional group of vagal afferents, the muscle mechanorecetors, activation of which can also induce emesis ((22); see also Chapter 11).

3) The view of the area postrema as the chemoreceptor trigger zone for emesis has in general been taken to imply, particularly in the case of cytotoxic drugs, that the area postrema responds directly to the drug or some cellular effect that the drug may have. However, it should be borne in mind that the area postrema could be responding to a substance released, for example from the gut mucosa, by the cytotoxic drug or radiation. The latter mechanism has been invoked to explain the emetic response to high-dose radiation that occurs after section of the vagus and greater splanchnic nerves (23, 24).

With area postrema ablation the problem is not so much of assessing what has been removed but more of what has been left behind. In addition, there is a need for histological studies in which orthograde tracers are used to map the distribution of abdominal vagal afferents in area postrema lesioned animals. Such studies should also be supported by functional studies of reflexes that are mediated via the nucleus tractus solitarius (25).

One question that arises from area postrema lesion studies is "how much of the area postrema is needed?" Wang and Borison (26) emphasized the importance of assessing the completeness of area postrema ablation by not only testing whether the

emetic response to central agents such as apomorphine given at the ED_{100} is abolished, but also ensuring that the response to a higher dose is abolished. This ensures that the lesion has not simply increased the threshold of the emetic response. In the study of the early phase of radiation-induced emesis in the dog (27), Wang *et al.* comment that "to prevent the early emesis it is not necessary to eliminate the *entire* (our italics) trigger mechanism." In the dogs concerned, the latency of the emetic response to a normal dose of apomorphine was increased from 2 to 14–17 minutes, whereas the early emetic response to radiation was abolished. They argue that more than half the emetic receptors in the area postrema must have been destroyed "because recent experiments have shown that extensive destruction of the trigger zone on one side did not materially increase the apomorphine threshold." Further studies in the dog by Brizzee *et al.* (20) also reported upon the effect of partial lesions. In nine animals that failed to vomit (in over 4 hours) in response to X-irradiation, five had total ablation of the area postrema and a variable amount of damage to the dorsal sensory nucleus, whereas in four the lesions were described as being relatively "light" and part of the area postrema remained. In four additional animals with "light" or superficial lesions of the area postrema, vomiting was abolished in one animal but occurred within the normal latency range in the remainder.

An additional difficulty with surgical ablation of the area postrema, particularly with cautery, is that the lesion is likely to damage the microvasculature of adjacent brain regions. This could contribute to the collateral damage reported, but perhaps of more interest it could prevent access of humoral emetic agents to the superficial subnuclei of the nucleus tractus solitarius, which together with the area postrema could have a chemosensory role.

Abdominal nerve lesions

Although surgical section of the abdominal vagi and greater splanchnic nerves is technically simpler than area postrema ablation, there are still problems with interpretation. Firstly, the lesion is intended to cut the afferent connections between the gut and the brain, but because the vagal and splanchnic nerves contain both afferents and efferents the lesion is not selective. The abdominal vagus is composed of 80–90% afferent fibres, whereas the splanchnics are ~50% afferent. Although it is well established that section of the vagus and splanchinic nerves does not interfere with the ability of the animal to vomit in response to a centrally acting emetic (e.g. apomorphine or loperamide (23, 25)) the abdominal visceral components of the response will be modified. Of greater significance is the impact that section of the efferents (conventional autonomic efferents and the axon-collaterals of the afferents) may have in modifying gut function. Studies in the ferret suggest that abdominal vagotomy is the trigger for the induction of a novel mechanism involved in the emetic reponse to high dose radiation (see below (23, 24)).

Care needs to be taken when reading some of the earlier literature as the effect of dorsal rhizotomy, usually between thoracic segments T_6–T_{10}, was also investigated

(27, 28). Although this procedure will certainly destroy abdominal splanchnic afferents (except those that may enter via the ventral roots) it will also de-afferent some somatic muscles involved in respiration. Although it is not thought that these are directly involved in initiating the emetic response, it could be modified if these afferents provided some feedback, for example regulating the number of retches/burst (29).

It is apparent from the above discussion that abdominal visceral nerve lesions are fraught with as many interpretational difficulties as area postrema ablation. For example, if the response to a cytotoxic drug or radiation is not blocked by the peripheral nerve lesion it could be because:

a) the stimulus is acting at another site (e.g. the area postrema)
b) the stimulus acts equi-effectively at either site
c) in the neurally intact animal the stimulus acts solely via the gut afferents, but when these are removed the system reorganizes (i.e. displays plasticity) and an alternative mehanism is induced or expressed (23, 24).

Because of the growing evidence for plasticity in the emetic pathway, it has become necessary to develop a technique for acute vagotomy in which the vagus can be cut immediately before administration of the emetic, or even during vomiting (see below).

Neurophysiological studies

This is probably our area of greatest ignorance in understanding the mechanisms by which anti-cancer therapies induce emesis. Technically, recording neural activity from the area postrema *in vivo* is difficult, partly because the neurones are small, and at least in the barbiturate-anaesthetized dog, appear to have no spontaneous activity that would aid identification (30). Because of this, they are identified by iontophoretic application of the excitatory amino acid glutamate, which raises the question of whether this may sensitize the neurones to subsequently applied stimuli. Carpenter *et al.* (31) have recorded from the area postrema in two dogs following irradiation, and reported the presence of low-level activity in the neurones and an increase in activity consistent with the time-course of emesis seen in conscious dogs. Although this is suggestive that radiation may release an agent into the blood which then acts on the area postrema, the neurones could also have been activated via vagal afferents. It would be useful to perform similar studies in animals treated with cisplatin, and under conditions of acute and chronic abdominal nerve lesions.

Neurophysiological studies of the area postrema have provided two insights into its physiology, which may assist in reconciling some of the apparent discrepancies in the literature on pathways discussed below. Firstly, Carpenter *et al.* (32) showed that many of the agents that activate area postrema neurones use cAMP as their intracellular second messenger. Systemic administration of agents that elevate cAMP (e.g. 3-isobutyl-1-methyl xanthine) reduced the threshold for the induction of emesis by apomorphine, angiotensin, leu-enkephalin and insulin. This raises the possibility that an emetic could have a primary site of action, for example in the gut, to activate afferents projecting to the area postrema/nucleus tractus solitarius, but

have an additional action on the area postrema whereby the threshold required for the vagal input to induce emesis is reduced. This would mean that area postrema ablation would remove the facilitatory influence and block emesis. Abdominal nerve lesions would also have the same effect because the primary drive to emesis is missing. This mechanism could also operate the other way around, with the gut providing the facilitatory input, and such a scheme was proposed in 1963 by Bhargava *et al.* (33) in connection with salicylate-induced emesis. Secondly, studies of the cardiovascular influences of the area postrema (34) have shown that its neurones respond to circulating vasopressin (ADH) with either an increase or a decrease in firing, with both effects being mediated via V_1 receptors. In addition, vasopressin, via an action on the area postrema, can increase the sensitivity of the baroreceptor reflex, which is mediated by vagal and glossopharyngeal inputs to the nucleus tractus solitarius (35). Both of these observations provide a strong indication that a modulatory role for the area postrema should be given proper consideration when mechanisms are reviewed.

Although there is no suggestion that the area postrema neurones are directly sensitive to radiation (although they may be activated by oedema of the brainstem caused by local radiation (6)), for cytotoxic drugs the possibility exists of both indirect and direct actions. Recordings have not been made from area postrema neurones in animals treated with cytotoxic drugs, although the expression of c-*fos* in the area postrema of vagotomized ferrets treated with cisplatin is suggestive of a direct effect (see Chapter 11). Electrophysiological studies in cultured dorsal root ganglion cells have shown that cisplatin (1–5 μM) can induce a transient increase in excitability (36, 37). Chronic (5–12 days) exposure resulted in complex effects that were suggestive that cisplatin interfered with cellular calcium homeostatic mechanisms. This may be of functional significance, as intracellular calcium appears to mediate the effects of angiotensin II and vasopressin on area postrema/nucleus tractus solitarius neurones in culture (38). Some of the effects (e.g. adverse effects on cell survival) of chronic exposure to cisplatin were reduced by dexamethasone (37). In the isolated rat vagus, cisplatin reduced the depolarization induced by 5-HT (mostly mediated by 5-HT_3 receptors), and this effect was itself reduced by dexamethasone (39). The precise relationship of these studies to emesis is unclear, but they clearly illustrate that at clinically relevant concentrations cisplatin can have direct effects on neural tissue.

Studies investigating the effects of cisplatin administration on discharge from abdominal vagal afferents are described in Chapters 5 and 8, and will not be reviewed here.

What is measured?

To characterize the emetic response fully it is necessary to know the number of animals responding (retching or vomiting) compared with those tested, the latency, the temporal profile of the response and the total number of retches and vomits in the observation period. Although the majority of recent studies provide such

information, many of the earlier ones do not, and this includes some of the critical studies of the pathways involved in radiation and cytotoxic drugs. The paucity of information makes it difficult to interpret such studies in the context of more recent findings. In addition many of the studies, particularly those involving dogs, cats, and monkeys, are highly unlikely to be repeated under more rigorous conditions.

An overview of the data

Bearing in mind the above technical problems and difficulties of interpretation, we can now review the experimental literature. For convenience, the results from each species will be reviewed separately, and confined to studies of radiation and cisplatin as these are the most extensively studied. Although cisplatin is used as an example of a cytotoxic drug it must be emphasized that although other cytotoxic drugs use the same general pathways, some may have additional pharmacological effects and sites of actions (e.g. the indirect cholinergic effects of nitrogen mustard on the cerebral cortex (40)). The effects of the various lesions on the emetic effects of apomorphine (acting via the area postrema), and copper sulphate (acting predominantly via abdominal afferents) will be discussed for comparison.

Monkey

The effects of area postrema ablation on the response to cisplatin have not been determined in any primate as far as we are aware but it is appropriate here to make a comment about the area postrema in primates. In humans, the emetic effect of apomorphine is abolished by area postrema ablation (17). However in two species of monkey (*Macaca mulatta* and *Macaca cynomologus*) apomorphine, and lanatoside C both failed to induce emesis (20) and lead the authors to conclude that the area postrema was "virtually non-functional". In another primate the common marmoset (*Callithrix jacchus*) tetralin (a dopamine D_2 receptor agonist) does induce emesis (41). Although the area postrema is not involved in motion-induced emesis, there appear to be differences in sensitivity, with the squirrel monkey being very sensitive but the macaque resistant (42). From these few studies it is difficult to make any firm conclusions about the functionality of the area postrema (if indeed it is where apomorphine acts rather than on the dendrites of nucleus tractus solitarius neurones projecting into the territory of the area postrema, see Chapter 11). The insectivores are the closest extant relatives to primates but the house musk shrew (*Suncus murinus*) does not have an emetic response to apomorphine or morphine (43, 44) although it possesses an area postrema and a potent emetic response to other stimuli, particularly motion (43). We should also remember that primates are a very diverse ancient group (at least 65 million years) and have a longer geological history than any other placental mammals except the insectivores and carnivores (both of which have sensitive emetic reflexes!). In the same way that differences in sensitivity to a range of emetic stimuli exist between carnivores we should not be surprised that amongst primates differences occur. Even different races of humans are reported to have a range of sensitivity to motion stimuli that do not appear to be culturally related (45).

Abdominal vagotomy (at least 7 days earlier) in cynomologus monkeys abolished the vomiting response to cisplatin in three animals tested, abolished retching in two animals, and the remaining animal had a very delayed response (46). The response to oral copper sulphate was markedly reduced and the latency doubled by abdominal vagotomy.

Area postrema ablation (which also included "a variable amount of damage to superficial portions of the dorsal sensory nucleus and the descending vestibular nucleus", and in one case damaged the dorsal motor nucleus of the vagus) abolished the emetic responses to total body X-radiation (1200 cGy, 21cGy/minute (20)) in 13 out of 14 animals. A similar result was obtained again by Brizzee (47) whose lesions also included "moderate damage to the dorsal sensory nucleus". He also investigated lesions directed at the dorsal sensory vagal nuclei and found that the lesion abolished emesis in two animals and delayed the onset in a third, however the lesions in the two protected animals "encroached heavily on the area postrema". Brizzee (47) also examined subdiaphragmatic (abdominal) vagotomy and found that it abolished the response to radiation. The discussion of this and other papers by Brizzee (48) contains many insights into the organization of the pathways for emesis in the medulla and the overview presented below draws heavily upon them.

Dog

Ablation of the area postrema abolished the emetic response to cisplatin (2, 3 and 6 mg/kg i.v.) and apomorphine (49, 50), but did not affect the initial response to oral copper sulphate (51). Section of the greater splanchnic nerves was without significant effect on the response to cisplatin, but when combined with abdominal vagotomy (> 7 days before test) the emetic response was abolished in the three animals studied (52). This combined lesion also abolished the emetic response to cyclophosphamide (20 mg/kg i.v.), actinomycin D (50 μg/kg i.v.), and nitrogen mustard (5 mg/kg i.v.). The emetic response to the latter was also abolished by area postrema ablation (53).

Abdominal vagotomy in combination with greater splanchnic nerve section markedly reduced the number of emetic episodes to copper sulphate given orally (11.0 ± 2.5 *versus* 1.3 ± 0.3), but only slightly increased the latency (0.27 ± 0.07 hour *versus* 0.37 ± 0.14 hour). This is somewhat different from the original observations of Wang and Borison (51) in which a similar (more extensive sympathectomy in combination with vagotomy) lesion abolished the response to a conventional dose (40 mg) of copper sulphate and when the animals did respond to a much higher dose (320 mg) the latency was about 2 hours.

In animals with area postrema ablation (demonstrated functionally by a lack of apomorphine response) all studies are unanimous in their conclusion that the acute or early response to whole-body radiation (600–1200 cGy, 16–83 cGy/minute) is abolished (27, 31, 54). The studies with abdominal nerve lesions are less extensive but are nevertheless also consistent. When tested at least 1 month and usually 2–4 months after abdominal vagotomy, animals responded with emesis (not

quantified) although at a longer latency (control 45.1 ± 22.2 minutes *versus* lesioned 104 ± 33.8 minutes). Whilst sympathectomy had a minimal effect on latency (68 ± 34.5 minutes) the combined lesion increased the latency further than vagotomy alone (129.7 ± 50.9 minutes), and in two out of nine animals the response was abolished (27). Combination of abdominal vagotomy with dorsal rhizotomy (T_6–T_{10}) was no more effective than vagotomy combined with splanchnic section. Carpenter *et al.* (31) reported results from two dogs in which the abdominal vagi were cut 60 days before testing. Both animals responded although the "average" latency was increased (control 102 minutes *versus* lesioned 148 minutes), and there was a reduction in the number of emetic episodes (control 7.4 *versus* lesioned 4). With such small numbers caution must be exerted in interpretation but the trend to increasing latency is similar to that in the more extensive study of Wang *et al.* (27).

Cat

Cisplatin reliably induces emesis in the cat at a similar latency to other species (55). The dose that has generally been used is 7.5 mg/kg, which is similar to that used in the ferret (56) and *Suncus murinus* (43), but about twice that in the dog (52), monkey (46) and pig (57; see Chapter 19). Area postrema ablation abolishes the response to cisplatin in the cat (19, 55), but appears to have little effect on the response to nitrogen mustard, phosphoramide mustard and cyclophosphamide, although there is a paucity of quantitative data (19, 58). In three cats with combined chronic abdominal vagotomy and splanchnectomy, the number of emetic episodes induced by cisplatin was unaffected (59). In contrast, in two animals, vagotomy combined with dorsal rhizotomy (T_5–T_{10}) blocked the response to nitrogen mustard (40).

The pathways involved in acute radiation-induced emesis have been more extensively studied, but it should be remembered that the dose of radiation required to induce emesis in the cat is substantially higher than in other laboratory species (~5500 cGy *versus* 125–800 cGy (6); see Chapter 12) as is the dose of apomorphine (~1000 times greater than the dog).

In 11 cats exposed to 4500 or 6000 cGy (head shielded, Co^{60} source, 450 cGy/minute) area postrema ablation abolished the emetic response (60). An earlier study by Borison *et al.* (61) using similar doses reported vomiting in four out of five area postrema ablated animals and in two out of three area postrema ablated animals in which only the gut was irradiated (4500 cGy X-rays, 500 cGy/minute), although in the latter the latency may have increased (27 minutes [values 4 and 63 minutes] *versus* 115 [values 46 and 184 minutes]). It is very difficult to reconcile these two discordant sets of results without invoking the explanation advanced by Borison (62) that the lesions in the Rabin *et al.* study (60) must have involved visceral afferent pathways. This explanation is certainly consistent with the effects of visceral denervation reported by Wang *et al.* (27), and Borison *et al.* (61). Cats in which the abdominal vagi had been sectioned in combination with a dorsal rhizotomy (more than 1 month before test (61, 63)) were exposed to a whole body

X-ray dose of 5500 cGy (210 cGy/minute) and showed an increase in the latency of the response (104.2 ± 84.9 minutes *versus* 265.8 ± 52.9 minutes). No information was given on the number of emetic episodes, and no statistical analysis was performed, but the range in the control group was 5–255 minutes whereas in the lesion group it was 175–315+, (with the meaning of "+" not given). Using a dose of 4500 cGy whole-body radiation, Borison *et al.* showed that abdominal vagotomy increased the emetic latency in two cats (66 minutes [range 3–129] *versus* 131 minutes [123–138]). Cordotomy (high superficial section of the dorsal columns) had a similar effect (latency 146 minutes [119–185]) but may in fact have been more effective as only five out of eight animals vomited. In three animals with combined vagotomy and cordotomy no emesis was induced. The authors concluded that the study provided further evidence that the area postrema was not involved in radiation-induced emesis in the cat but that the vagus, sympathetic, and 'co-sympathetic' afferents were involved, with the latter perhaps playing a substantial, but previously unrecognized role. We are not aware of any other studies that have investigated the effects of cordotomy on the emetic reflex.

Ferret

The ferret responds to cisplatin given either intraperitoneally (i.p.) or intravenously (i.v.) at a dose of 7.5–10 mg/kg, which is comparable to the doses used in the cat (see above). Section of the abdominal vagi and greater splanchnic nerves (more than 7 days before test) completely protected three out of four animals against the emetic effects of intraperitoneal cisplatin (10 mg/kg) with the responding animal having 16 retches but no vomits (64). Section of the greater splanchnic nerves alone was without effect, but vagotomy blocked vomiting in four out of five animals. All animals retched, although the pattern was modified, with retches being distributed throughout the observation period (5 hours) rather than being concentrated in the first 2 hours. Using intravenous administration of cisplatin (10 mg/kg) Davis (65) showed that the entire emetic response could be abolished by vagotomy alone. A similar effect was reported by Kamato *et al.* (66) with two out of three animals being completely protected by vagotomy alone and the third only showing retching. Greater splanchnic nerve section alone did not protect any animals but did reduce the number of retches and increase the latency. The combined lesion of vagotomy and greater splanchnic nerve section completely protected a group of four animals over the 6 hour observation period. From these observations, it appears easier to block the emetic response to cisplatin delivered intravenously than intraperitoneally, and this also appears to be the case with cyclophosphamide (64). Previous studies in the ferret have also demonstrated marked reductions in the emetic response (including complete protection) to cycloheximide, mustine, diacetoxyscirpinol and emetine (23, 65).

Area postrema ablation has been performed in the ferret, and the lesion abolished the response to the centrally acting opioid receptor agonist loperamide. The emetic response to cisplatin (10 mg/kg i.p.) was abolished by area postrema ablation

(Watson, Kovacs and Andrews, unpublished observations). However, so was the response to copper sulphate given orally, although retching could still be induced by electrical stimulation of abdominal vagal afferents. The observation with copper sulphate is interesting, as in their human study Lindström and Brizzee reported a similar finding in one of their patients (17).

Of the species studied to date, the ferret is the most sensitive to the emetic effects of radiation with the ED_{100} being ~125 cGy (65). Studies on the effects of nerve lesions have been carried out using three different doses of radiation and this has provided new insights not revealed by previous studies employing a single dose (23, 24, 65, 67). In addition, both neutrons (68) and X-rays (23, 24, 65) have been investigated. After 200 cGy X-rays, chronic abdominal vagotomy (7–10 days before the test) abolished retching in five out of seven animals, and vomiting in six out of seven, with the response in the responders being reduced and delayed (23, 24). In contrast, exposure to 800 cGy following vagotomy resulted in all animals responding although the latency was increased from 17.2 ± 0.7 minutes to 33.6 ± 3.8 minutes, and the number of retches and vomits decreased by ~20–30% (23, 24, 65). Combination of vagotomy with greater splanchnic nerve section did not confer any additional benefit. Other studies using 400 cGy delivered from a cobalt source to head-shielded animals obtained similar results to those with total body exposure to 800 cGy X-rays (67). Initially we thought that this indicated that at 200 cGy the pathway was predominantly vagal, whereas at higher doses a vagal and a humoral mechanism was involved. However, because 5-HT_3 receptor antagonists were quite effective against emesis induced by 800 cGy in intact animals (whereas they were relatively ineffective in vagotomized animals) it appeared possible that a novel mechanism came into play once the vagus was sectioned (23, 24, 65). We proposed that in vagally intact animals the entire emetic response was mediated by the vagus, irrespective of the radiation dose. Section of the vagus induced the formation or expression of a humoral agent that could be released by a 'high' dose of radiation, or perhaps a lower one in sensitive individuals. Its release may also depend upon the nature of the radiation, as vagotomized animals exposed to 200 cGy of radiation with a neutron:gamma ratio of 6:1 have a similar response to the animals exposed to 800 cGy X-rays (23, 24, 68). If the vagus mediates the response to 200 cGy and 800 cGy then section of the vagus immediately before radiation exposure should abolish the response, as the novel mechanism will not have had time to be induced. Therefore, a preparation was developed (24) in which the ventral abdominal vagal trunk and both greater splanchnic nerves were sectioned at one operation and the dorsal vagal trunk mobilized so that it could be cut without further abdominal surgery via a fistula. Our previous studies and those of others (67) had shown that either vagus could support the entire emetic response. One week after the surgery the dorsal vagus was cut and the animals (n = 4) immediately exposed to either 200 or 800 cGy. No responses were seen in either group following this acute vagotomy supporting the above hypothesis. Using this preparation we have also demonstrated that section of the vagus during emesis in response to 800 cGy stops the emesis

immediately, an effect reminiscent of that produced by intravenous injection of a 5-HT_3 receptor antagonist.

Area postrema ablation abolished the emetic response to both 200 and 800 cGy in animals tested at least 7 days after surgery (Andrews and Bhandari, unpublished observations). The effect of area postrema ablation in animals with chronic abdominal vagotomy has not been investigated but we predict that it would abolish the response.

Suncus murinus

There have not been any reports of the effects of area postrema ablation on the response to any emetic stimulus in *Suncus murinus*, and as this species fails to respond to apomorphine or morphine, some other agent with a central effect (possibly nicotine) will need to be used as the test stimulus.

The emetic response to cisplatin was completely abolished by abdominal vagotomy (69). The ED_{50} for whole-body X-irradiation in *Suncus murinus* is calculated at 429 cGy. Head irradiation did not induce emesis whereas abdominal irradition was effective, and the emetic response to whole-body X-radiation (800cGy, 66–70 cGy/minute) was abolished by abdominal vagotomy (70).

AN ATTEMPT AT A UNIFYING HYPOTHESIS

From the above species-based descriptions of the effects of the various lesions on the emetic response to radiation and cytotoxic drugs (particularly cisplatin), there appear to be differences in the pathways involved. Although they have been recognized from the time of the earliest cat and dog studies, and Borison drew particular attention to them in his 1989 review of the area postrema (62), sometimes they have been rather dismissively ascribed to species differences. If there are real species differences then we should exploit them to increase our understanding of mechanisms.

In an attempt to reconcile and explain some of the apparent differences Harding (71) proposed the idea of 'predominant pathways'. In essence this hypothesis proposes that all the species concerned have the two major pathways, the area postrema and the abdominal visceral afferents (mainly vagal), but in some species the stimuli act predominantly via one route rather than the other. This hypothesis is very appealing and indeed it is difficult to think of viable alternatives. However, we should ask what advantages would be conferred by using one pathway over another, and is it reasonable to expect that the 'odd' species in terms of pathways are really that different?

In this section we will take a broad overview of the above studies using a quotation from the preface to S.J. Gould's book *Wonderful Life – The Burgess Shale and the Nature of History* as a guide "The beauty of nature lies in the detail; the message in generality". In addition we will look at the species perhaps in a more

zoological context than has previously been done. We should remember that each species has physiological and other mechanisms that best equips it to survive in its environment. Although the focus is on identification of the species that is the best model for humans, those that appear more distant should not be regarded as 'wrong', or more importantly, irrelevant to our understanding of mechanisms. They may represent a more primitive or fundamental state that indicates something of the original mechanism.

Several issues need to be discussed before presenting an hypothesis:

What is the phylogenetic relationship beween the various species?

Although the emetic reflex is widespread throughout the vertebrates, attention has naturally focused on mammals. Of these, currently just a handful of species has been used to investigate the mechanisms of emesis in anti-cancer therapy, and a few more to investigate general emetic mechanisms. This relates to availability of species for laboratory investigation rather than lack of zoological knowledge (see Chapter 1). Of the currently studied species apart from humans we have one primate, the macaque (Old World monkey); an insectivore, the house musk shrew *Suncus murinus* (the oldest group of eutherians and the closest group to primates); and three carnivores (dog, cat and ferret). The major controversy lies within the carnivore group with the dog and ferret on the one hand and the cat on the other.

Which is the odd species?

This can be looked at from a zoological perspective and also from the point of view of similarities and differences in the effects of the various lesions, although caution must be exerted in view of the small number of species studied.

The dog (Canoidea) and cat (Feloidea) branches separated from the first true carnivores, the miacids, about 55 million years ago, with the dog branch arising from the vulpavines of the New World, and the cat branch from the viverravines of the Old World (72). The mustelidae, of which the ferret is a typical representative, is the most recent offshoot of the dog branch diverging about 33 million years ago. In view of this phylogeny, it would not be unreasonable to expect the cat to differ in some ways from the dog and ferret but is it reasonable that the cat should employ a different pathway?

If the results from all the species are examined from a broad perspective then we believe that there is a clear and consistent pattern that unifies rather than divides.

What does area postrema ablation do?

Whatever the technical issues (see above) surrounding the lesion, whenever the effects of area postrema ablation have been studied, the usual response is abolition of the *acute* emetic response to radiation and cytotoxic drugs (e.g. cisplatin). Studies in which mixed responses are reported (e.g. Brizzee *et al.* (20)), some members of the group are protected, whereas others have an increased latency. All studies show, therefore, that area postrema ablation has the *potential* to abolish the response.

What is destroyed by area postrema ablation?

The answer to this question is probably the key to resolving the apparent discrepancies. With the relatively crude techniques used to make the lesion it is inconceivable that collateral structural damage does not occur, surrounded by a penumbra of functional damage, in which some cells may be capable of recovery ('idling'), and some may be dying, depending on the time at which emesis is tested. Even using conventional histology there are considerable variations in the extent of damage reported. The lesions are made by using the surface projection of the area postrema as a guide, but this takes little account of the three dimensional organization of the underlying structures. The importance of this was recognized by Laffan and Borison (73) who in 1957 provided this insight on species differences:

> A plausible explanation for the species difference is that the centripetal emetic pathways are more tightly funnellled through the region of the CT zone in the dog than in the cat. Thus a discrete lesion of the CT zone of the dog would be expected to interrupt more emetic afferents of different origin, local and distant, than it would in the cat.

In this quotation it is important to note that they refer to emetic afferents of different origin rather than just vagal afferents.

The area postrema itself is usually viewed as the site at which transduction of the emetic stimulus occurs. However, dendrites from the nucleus tractus solitarius also project into the area postrema (74) and if these elements are actually the site at which apomorphine, opioids, and endogenous emetics (released by radiation and cyotoxic drugs) act, then the area postrema is only providing a window for access. In addition, as the dendritic tree of the nucleus tractus solitarius neurones is likely to be the main site for visceral afferent termination (Chapter 11) then area postrema ablation is also likely to damage such inputs either directly or indirectly (e.g. compromised blood flow).

What is being proposed here is that the conventional view of the area postrema is incorrect, and as far as lesion studies are concerned other interpretations are more consistent with the effects of the lesions being caused by collateral damage. This view we believe is also consistent with the effects of visceral nerve lesions dsicussed below.

Does the area postrema have a role in emesis?

The answer is "yes". Firstly, that the area postrema and associated dorsal vagal complex have a general chemosensory role cannot be denied, even if we cannot define the function of the various components (62). In this context, it is highly likely that the emetic response seen in animals after abdominal visceral nerve lesions is mediated centrally, although the relative degree of involvement of the area postrema and nucleus tractus solitarius remains to be elucidated.

Secondly, one view of the area postrema is that because of its close relationship to the nucleus tractus solitarius (the major integrative nucleus for visceral information, particularly vagal) it has a major role as a modulator of information transfer through

this nucleus. This is a very attractive role as the vagus is viewed as the major 'interoprotective' nerve of the epithelia, and the largest and fastest changes to plasma composition are likely to arise from breathing, drinking and eating (75).

We would envisage the area postrema as being able to sensitize the emetic reflex. Under what conditions and how this occurs (32) is not clear but the genesis of conditioned taste aversion may be an analogous process.

What is the role of the abdominal visceral afferents?

From the above discussion it is apparent that, if we ascribe a minor role to the area postrema, then the visceral afferents, particularly those from the abdomen, must have a major role. Interpretation of the often semi-quantitative data from many of the early studies is complicated by the growing recognition that the abdominal lesions may induce some form of plasticity, which may only be expressed at higher doses of radiation or equivalent chemical stimuli. If we take a general overview of the chronic abdominal lesion studies, it is clear that vagotomy has an effect that still causes a shift in the latency even when it does not constitute complete protection. Although section of the greater splanchnics alone is ineffective, their lesion always enhances the effect of vagotomy. The reason is not known but is likely to reside in their interactions in the brainstem. The studies in the cat are complicated because in this species it apears that there might be an additional pathway from the abdomen and thorax via the superficial dorsal columns. Lesion of this pathway in the cat enhances the effect of the other lesions. The nature of this pathway is unknown, but it may contain projections from the Pacinian corpuscles located in the mesentery in the cat. The effects of cordotomy need to be examined in other species before the significance of this pathway can be assessed. We propose that the abdominal afferent system is predominant in all species.

CONCLUSION

In this review we have attempted to provide an overview of the pathways by which cytotoxic drugs and radiation induce emesis. We suggest that many of the apparent discrepancies in the literature can be explained by technical difficulties, and in particular those associated with interpretation of area postrema ablation. Others can perhaps be accounted for by the plasticity of the emetic pathways, which probably explains the apparent lack of effect of abdominal nerve lesions in some studies. The problems of interpretation are compounded by the lack of quantification of all aspects of the responses in some earlier studies. Some of the key features of the pathways we currently believe are involved in the emetic response to cytotoxic drugs and radiation are summarized in Figure 4.

Although understanding of the mechanism has increased considerably in the last 10 years, driven to a large extent by the need to identify the site and mechanism of action of the 5-HT_3 receptor antagonists, many questions remain; how does limb

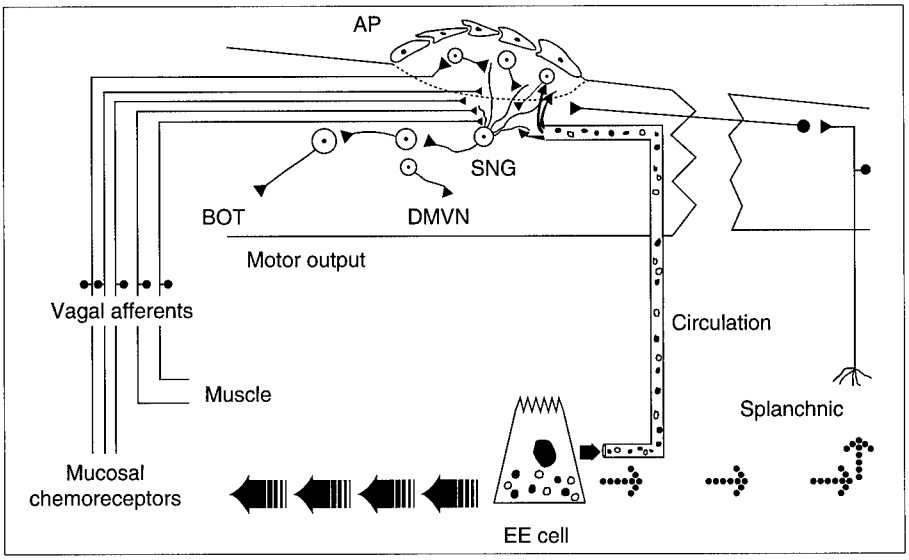

Figure 4. A diagram summarizing some of the key features of the pathways involved in the acute emetic response to cytotoxic drugs and radiation (see text for details and references). The motor outputs for vomiting are represented by the dorsal motor vagal nucleus (DMVN) for the vagal outputs to the gut, and the Bötzinger (BOT) complex for the somatic neural output to the diaphragm. The subnucleus gelatinosus (SNG) of the nucleus tractus solitarius is seen as a focal point for integration of the reflex, and neurones in the SNG have dendrites projecting into the area postrema (AP). It is proposed that a gradient of permeability in the blood-brain barrier exists in this dorsal brainstem region such that agents in the blood (e.g. apomorphine, gut peptides) can access the AP (and perhaps superficial dendrites of the SNG) to trigger emesis. Abdominal vagal afferents project to this region and we propose that the afferents from the mucosal chemoreceptors take a more superficial route and hence are more likely to be damaged by a lesion directed at the AP. At their site of origin in the gut wall the mucosal receptors are activated by chemicals (e.g. 5-HT, SP, CCK-8) released locally from entero-endocrine (EE) cells in response to cytotoxic drugs and radiation as well as their natural stimuli (e.g. mucosal stroking and hypertonic solutions). These cells are probably also the source for the endogenous emetic agent, which is probably responsible for the emetic response in the absence of the extrinsic abdominal innervation. The role of the splanchnic afferents is unclear, but they could be affected by agents from the EE cells and via projections to the brainstem modulate the emetic reflex.

irradiation induce emesis, what determines species emetic sensitivity to radiation and cytotoxic drugs, what is the role of the splanchnic afferents, what is the nature of the endogenous emetic agent involved in the response to high dose radiation following abdominal nerve lesions and how is it released? Such questions will provide a fertile area for research into the next millenium and the answers may further improve the care of patients undergoing cancer therapy.

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Chapter 3

The discovery of selective 5-hydroxytryptamine-3 (5-HT_3) receptor antagonists

B. P. Richardson

EARLY EVIDENCE FOR 5-HT RECEPTOR SUBTYPES

More than 40 years ago, Rocha e Silva and his colleagues in Sao Paulo, Brazil made the important observation that cocaine selectively inhibits the contractile action of 5-HT on the guinea pig ileum (1). They suggested that this results from the ability of cocaine to block the action of 5-HT at receptors located on postganglionic cholinergic neurones. In parallel with these studies, Gaddum and Hameed in Edinburgh suggested that 5-HT might, in fact, be activating two different receptors in the guinea pig ileum, one of which was located on the smooth muscle and the other on nerves (2). Ultimately, a representative from each of the two teams collaborated on what is now acknowledged to be a seminal series of experiments. Gaddum and Picarelli performed a quantitative analysis of the contractile action of 5-HT on the guinea pig ileum and confirmed the existence of two pharmacologically distinct 5-HT receptors. They found that dibenzyline could antagonize the direct action of 5-HT on smooth muscle, whereas morphine could block its indirect contractile action mediated through activation of neuronally located receptors. Because of the sensitivity of the 5-HT receptors located on the smooth muscle to dibenzyline and those on neuronal elements to morphine, they named them 'D' and 'M' receptors, respectively (3). Not only was this the first time that different subtypes of receptors for 5-HT had been conclusively demonstrated, but it was Gaddum and Picarelli's M receptor that was ultimately renamed the 5-HT_3 receptor some 30 years later (4).

5-HT RECEPTOR CLASSIFICATION

For more than 20 years, the 'D' and 'M' nomenclature survived, despite the obvious short-comings of the pharmacological tools used to define them – dibenzyline being an alkylating agent with high affinity for α adrenoceptors and morphine and cocaine having well recognized actions at opiate receptors and neurotransmitter uptake sites, respectively. Finally, in 1979, Peroutka and Snyder identified two distinct binding sites for 5-HT in rat brain using radioligand binding techniques. The high affinity site was named the $5HT_1$ site and the low affinity site the 5-HT_2 site (5). For a short period of time the D/M and 5-HT_1/5-HT_2 nomenclatures existed side by side, but it

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was soon realized that the D receptors on smooth muscle were pharmacologically identical to 5-HT_2 binding sites. However, 5-HT_1 binding sites were clearly different from neuronal M receptors, as was demonstrated when potent selective antagonists for the latter became available (6, 7). Consequently, in 1986, Bradley *et al.* proposed a new classification of 5-HT receptors, in which the D receptors of Gaddum and Picarelli were designated 5-HT_2 receptors and the M receptors were designated 5-HT_3 receptors (4).

Since this reclassification, many more new 5-HT receptor subtypes have been identified (8). There are now seven recognized classes of 5-HT receptor (5-HT_1–5-HT_7) with several subtypes within the 5-HT_1 and 5-HT_2 classes. One of the main reasons for such rapid progress has undoubtedly been the discovery of selective agonists and antagonists for several of these receptor subtypes. The availability of such compounds for 5-HT_3 receptors has made a major contribution to the understanding of the neuronal action of 5-HT, and a personal account of how the Sandoz compounds were discovered is given below.

THE RACE FOR SELECTIVE 5-HT_3 RECEPTOR AGONISTS AND ANTAGONISTS

Our main competitor in the 'race' for the first highly potent 5-HT_3 receptor agonists and antagonists was Fozard and his team who, at the time, were working at The Merrell International Research Centre in Strasbourg. A detailed account of the events leading up to the discovery of the Merrell Dow 5-HT_3 receptor antagonist, MDL 72222, and its analogues has already been published (9). Essentially, the potent and selective 5-HT_3 receptor antagonist properties of MDL 72222 and ICS 205-930 from the author's group at Sandoz were presented to the British Pharmacological Society within 6 months of each other (10, 11). Other companies, such as Beecham and Glaxo, produced second-generation 5-HT_3 antagonists that were described in the literature about 3 years later (12, 13). Details of some of the key events and milestone dates in the discovery of the first four 5-HT_3 receptor antagonists are given in Table 1; details of their *in vitro* and *in vivo* potency for 5-HT_3 receptors, and their anti-emetic properties are provided in Table 2. Subsequently, there have been a very large number of potent, selective 5-HT_3 receptor antagonists described by different pharmaceutical companies (14). However, the potential clinical advantages of these compounds over the first and second generations of potent, selective 5-HT_3 receptor antagonists are not obvious.

The Sandoz initiative

In the mid 1970s, Lowe, Donatsch and I were studying the calcium antagonistic properties of the 5-HT_2 receptor antagonist cyproheptadine (15). We found that the compound could block voltage-sensitive Ca^{2+} channels in smooth muscle cells at

Table 1. Some key events and dates in the discovery of selective 5-HT_3 receptor antagonists.

Key scientists	Biological starting point	Chemical starting point	Start of chemical programme	Priority date (patent)	Resultant 5-HT_3 receptor antagonist	First publication	Compound class
J.R. Fozard M.W. Gittos (Merrell)	Action of 5-HT on rabbit heart	Meto-clopramide and cocaine	1978	6/1981 (EP067770)	MDL 72222 (Bemesetron)	1983 (10)	Benzoic acid tropine ester
P. Donatsch G. Engel B.P. Richardson P.A. Stadler (Sandoz)	Action of 5-HT on rabbit vagus nerve	5-HT	1979	7/1982 (UK2125395)	ICS 205-930 (Tropisetron)	1984 (11)	Indole-3-carboxylic acid tropine ester
I.H. Coates D. Humber M.B. Tyers S.J. Ireland (Glaxo)	Action of 5-HT on rabbit nodose ganglion	Indolyl-propanones	1979	1/1984 (UK2153821)	GR 38032 (Ondansetron)	1987 (12)	Imidazolyl-methyl-carbazolone
C.S. Fake F.D. King G.J. Sanger (Beecham)	Anti-emetic effect of metoclopramide and MDL 72222 in ferrets	ICS 205-930	1984	4/1985 (EP200444)	BRL 43694 (Granisetron)	1987 (13)	Indazole-3-carboxamide

Table 2. Potency of 5-HT_3 receptor antagonists on various biological systems (7, 9).

	In vitro (pA_2 values)			*In vivo*	
Compound	Vagus[a]	Heart[a]	Ileum[b]	Von Bezold-Jarisch Reflex (IC_{50} µg/kg i.v.)[c]	Cisplatin emesis (effective dose range µg/kg i.v.)[d]
MDL 72222 (Bemesetron)	7.9	9.3	6.7	39	0.1–1.0
ICS 205-930 (Tropisetron)	10.2	10.6	7.9	0.35	0.01–0.1
GR 38032 (Ondansetron)	9.4	10.1	7.3	0.42	0.01–0.1
BRL 43694 (Granisetron)	9.9	10.7	8.1	0.70	< 0.01

[a]Rabbit; [b]Guinea pig; [c]Rat; [d]Ferret

concentrations only slightly higher than those required to block the actions of 5-HT. In order to establish whether the blockade of Ca^{2+} channels was merely a reflection of generalized membrane stabilization, we investigated the ability of cyproheptadine to reduce the amplitude of the compound action potential in the rabbit isolated vagus nerve. We found that cyproheptadine possessed generalized membrane stabilizing properties in this preparation at concentrations greater than 5×10^{-6} M, which were approximately 50 times higher than those needed to block Ca^{2+} channels (15). In 1978, Neto reported that 5-HT could depolarize 'C' fibre afferents in the vagus

nerve and reduce the amplitude of the 'C' fibre action potential. He also showed that this was partially blocked by cyproheptadine at concentrations above 10^{-5} M, but not by methysergide, and concluded that this action of 5-HT was probably not mediated through specific receptors (16). Realizing that these concentrations of cyproheptadine produced generalized membrane stabilization, we re-examined the action of 5-HT on the rabbit vagus nerve by testing a large number of the then available antagonists (cinanserin, mianserin, methysergide, methiothepin, bromolysergide acid diethylamide). None of them could block the action of 5-HT to reduce the amplitude of the 'C' fibre action potential in this preparation, so we concluded that if the action of 5-HT was indeed receptor-mediated, we must be dealing with a receptor that was different from that previously described in a variety of smooth muscle preparations. A review of the literature then drew our attention to the earlier work of Gaddum and Picarelli, in which they described neuronal receptors in the guinea pig ileum that were also resistant to blockade by the classical ergot-based 5-HT antagonists (3). It was at this point, in the autumn of 1979, that we decided to characterize the excitatory action of 5-HT on peripheral neurones in more detail. A team was formed, consisting of Donatsch, Engel, Stadler and myself. Our first concern was to demonstrate that the depolarizing response reported by Neto was really receptor-mediated. In the absence of selective antagonists, we decided that the simplest way to confirm a specific receptor-mediated effect might be to synthesize a series of conformationally restricted analogues of the natural agonist, 5-HT, based on the assumption that different conformations of the 5-HT molecule would be necessary to activate D (5-HT_2) and M (5-HT_3) receptors. By systematic methyl substitutions in the indole nucleus and ethylamine side chain, we attempted to add steric hindrance and to reduce the conformational freedom of the 5-HT molecule, and thereby create analogues with increased specificity for one of these receptors.

This strategy indeed proved to be highly effective. Our chemist, Dr Stadler, being very systematic, started by synthesizing the 1-methyl analogue. As can be seen from Figure 1, this compound retained agonist potency on both D and M-receptors and thus did not differentiate between them. However, the second compound synthesized, 2-methyl-5-HT, was 2000 times less potent than 5-HT at D-receptors but still retained 50% of the potency of 5-HT at M-receptors. This compound therefore had 1000 times more selectivity for the M-receptor than the natural agonist. Conversely S(+) α-methyl-5-HT, the fourth compound synthesized, retained 50% of the potency of 5-HT at D-receptors in the vagus nerve but was almost 800 times less potent on M-receptors in the vagus nerve. Already, in the spring of 1980, this information was sufficient to convince us that the depolarizing action of 5-HT on the rabbit vagus nerve and on nerves in the guinea pig ileum was indeed mediated by specific receptors, which were different from those located on smooth muscle cells in the guinea pig ileum or the rat uterus. At around the same time, Peroutka and Snyder published their paper on 5-HT_1 and 5-HT_2 binding sites in rat brain (5) and we were able to show that 2-methyl-5-HT lacked appreciable

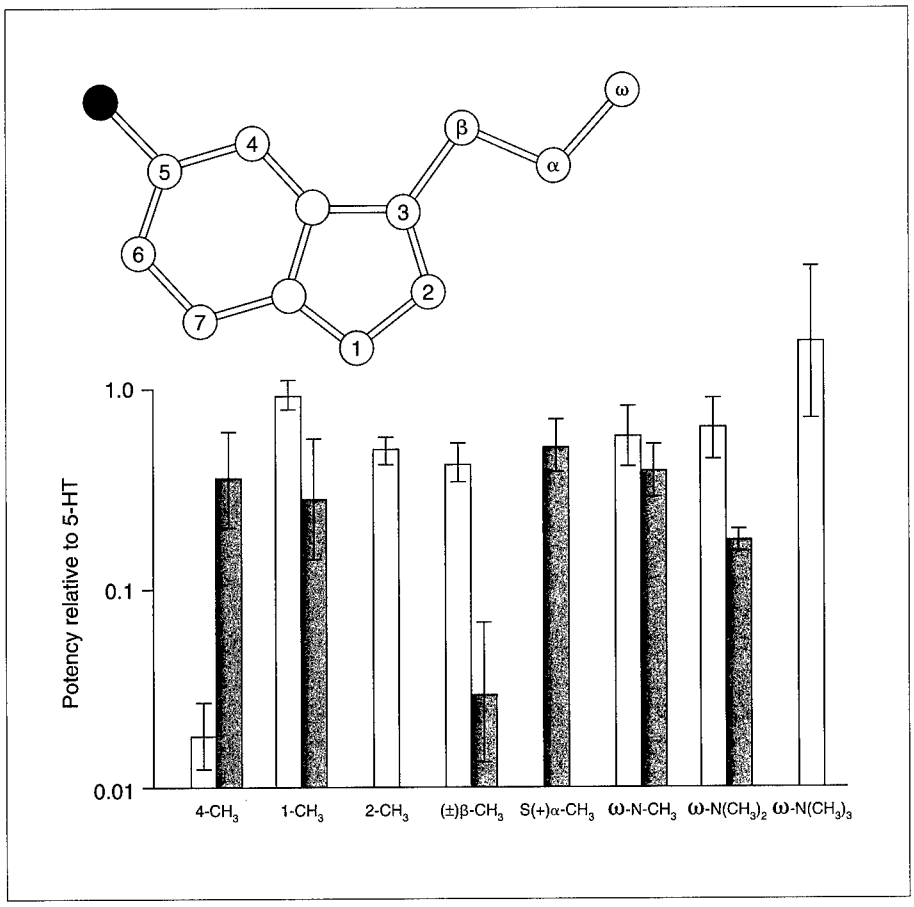

Figure 1. Agonist potencies of methylated serotonin (5-HT) analogues relative to 5-HT (potency = 1.0) on 5-HT_3 receptors in the rabbit vagus nerve (open columns) and 5-HT_2 receptors in the rat uterus (shaded columns). Each column indicates the geometric mean value obtained from at least three independent experiments. Vertical lines on each column indicate 90% confidence limits. Reproduced with permission from (7).

affinity for either of these sites, whereas S(+) α-methyl-5-HT bound to 5-HT_2 but not to 5-HT_1 sites. This provided clear evidence that the M receptors located on peripheral nerves were different from both 5-HT_1 and 5-HT_2 receptors found on central neurones.

Because of the potential shortcomings of classifying receptor subtypes based on the rank order of agonist potencies (17), we decided to embark on a programme aimed at producing selective competitive antagonists. We attempted to synthesize analogues of 5-HT that retained their selectivity for M-receptors but that lacked

intrinsic activity. Towards the end of 1978, Fozard and Mobarok Ali published data showing that metoclopramide competitively antagonized the action of 5-HT at receptors located on sympathetic neurones of the rabbit heart (18). This suggested the possibility of extending the ethylamine side chain of 5-HT in order to reduce intrinsic activity. When the indole ring structure of 5-HT was combined with the N-diethylaminoethyl aminocarbonyl side chain of metoclopramide a competitive antagonist with a pA_2 value of 7.3 was obtained (Figure 2). By synthesizing more rigid analogues where the terminal nitrogen atom in the side chain was included in various ring structures, a considerable increase in potency was obtained. The most potent compounds of this series were the tropine and homotropine analogues with pA_2 values greater than 9. Replacement of the amide group in the side chain by (formally) an ester, (more exactly a vinylogous urethane) further increased potency by a factor of 10. This resulted in (3α-tropanyl)-1H-indole-3-carboxylic acid ester, or ICS 205-930 (tropisetron), which was selected for further pharmacological characterization in a variety of *in vitro* and *in vivo* systems. This compound proved to be a highly potent and selective 5-HT_3 receptor antagonist, and was used to demonstrate the presence of 5-HT M-receptors (now 5-HT_3 receptors) in humans for the first time (7). This compound was developed for the prevention of cytotoxic drug- and radiation-induced nausea and emesis, and is now marketed for these indications.

The Merrell initiative

As indicated in Table 1 the programme to discover 5-HT_3 receptor antagonists at Merrell by Fozard's group ran in parallel with the activities at Sandoz (Table 1), and a full account of their approach has been published previously (9). The programme was based on a long-standing interest of Fozard in the 5-HT antagonist actions of cocaine and its derivatives on the rabbit heart. Consequently (–)-cocaine was used as the chemical starting point and, as shown in Figure 2, systematic substitution in the tropine and benzine rings ultimately lead to MDL 72222 (bemesetron), which underwent extensive *in vitro* and *in vivo* characterization. MDL 72222 was also investigated in humans and shown to block 5-HT_3 receptors in healthy volunteers as well as to provide relief of acute migraine attacks (9). The development of this compound was ultimately suspended for unknown reasons.

The Glaxo initiative

A full account of the discovery of GR 38032 (ondansetron) was published in 1992 (19). The lead compound, S37336-2 (Bader catalogue), was obtained by screening a large series of indole analogues for their ability to inhibit 5-HT_3 receptors in the rat vagus nerve (Figure 2). The indolylpropanone S37336-2 has a pA_2 value of 6.5. By systematic substitution of the tertiary amino group in the side chain, it was ultimately shown that the N-imidazolyl compound produced an unexpected increase in potency. Subsequently, it was shown that introducing a methyl group at the 1-position of the indole ring in this series produced only small changes in activity

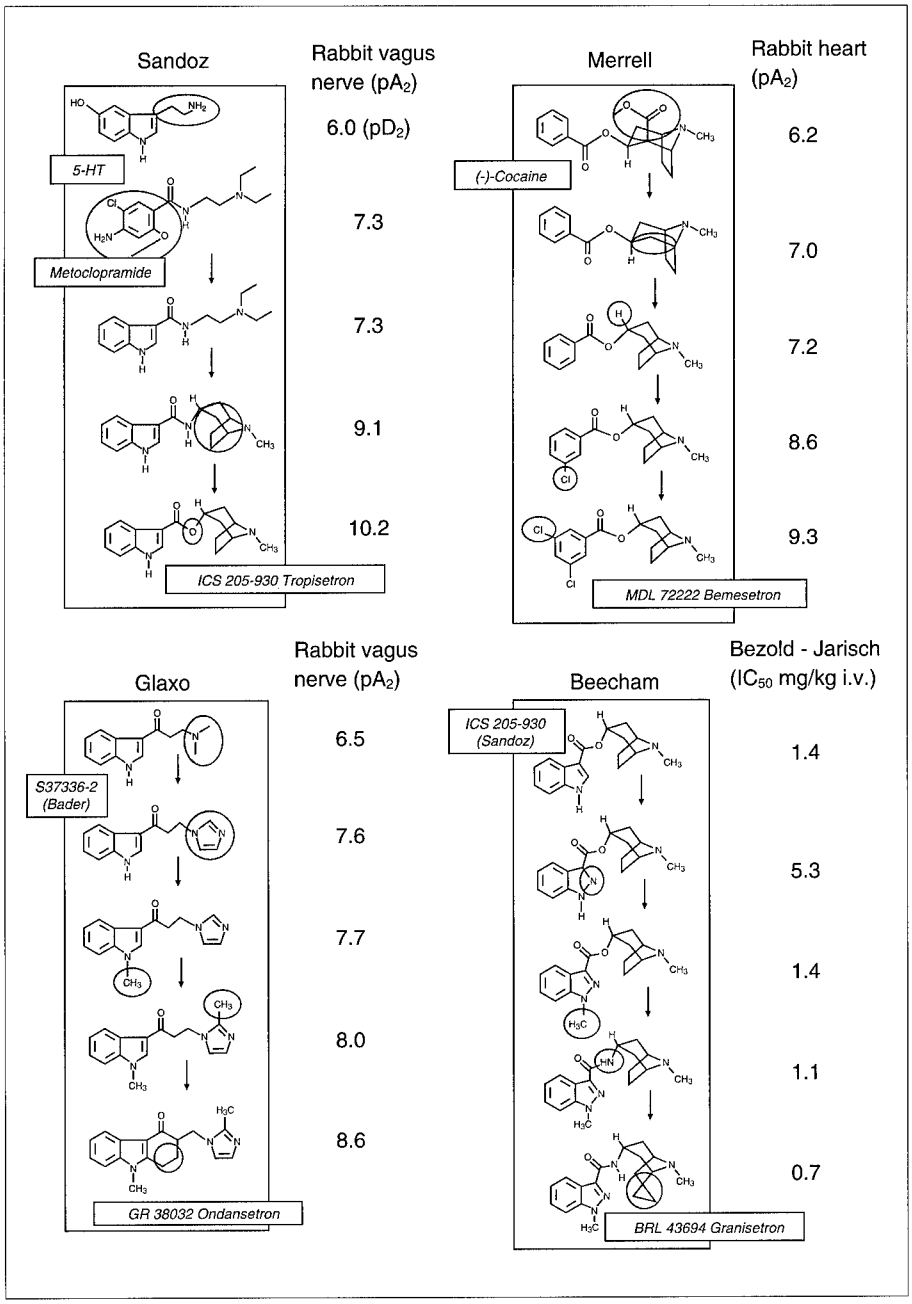

Figure 2. Different chemical strategies pursued by pharmaceutical companies that led to the synthesis of the first four 5-HT_3 receptor antagonists.

(Figure 2). Additional substitution of a methyl in the 2-position of the imidazole ring produced a further small increase in potency and at the same time avoided inhibition of the hepatic cytochrome P-450-linked oxidase enzyme system. Finally, incorporating the side chain into a tetrahydrocarbazolone system led to a further improvement in potency and to the orally active compound GR 38032 (ondansetron). This compound was extensively studied in *in vitro* and *in vivo* systems. It was investigated in several potential clinical indications and was developed for the prevention of cytotoxic drug- and radiation-induced nausea and vomiting. This compound is now marketed for these indications as well as for postoperative nausea and vomiting.

The Beecham initiative

The group at Beechams had been studying the anti-emetic and gastrointestinal promotile actions of metoclopramide and other structurally related benzamides for a considerable period prior to the emergence of selective 5-HT_3 receptor antagonists. The main aim of their efforts was to remove the dopamine D_2 antagonistic effects of such compounds in order to avoid extrapyramidal side-effects. Indeed, they were successful with BRL 24682, which retained both anti-emetic and promotile effects of metoclopramide, yet lacked its dopamine receptor blocking action (20). This compound was ultimately shown to possess both 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist effects that were responsible for its anti-emetic and promotile actions, respectively.

The search for selective 5-HT_3 receptor antagonists at Beechams began only after publication of the structure–activity relationships of ICS 205-930 analogues, which was presented by my colleague Dr Giger at the VIIth International Symposium on Medicinal Chemistry held in Uppsala in the summer of 1984 (21). The increased potency achieved by substituting a methoxy group in the 2-position of the indole ring of ICS 205-930 indicated to the Beecham group that a heteroatom at this position with a lone pair extending into space could provide an alternative acceptor site for intramolecular hydrogen bonding. Indeed, replacing the indole moiety by an indazole was tolerated with only a slight loss of potency (Figure 2). Substitution with a methyl group in the 1-position of the indazole ring produced a further improvement in potency, and concerns that tropine esters connected to indazole might be subject to metabolic degradation by serum or hepatic esterases led to replacement of the ester bond by an amide. In contrast to the indole series, this did not reduce potency (14). Finally, the tropine ring was replaced by a homotropine (granatane) ring, which provided a small additional increase in potency. The resulting compound, BRL 43694 (granisetron), has been extensively studied in both *in vitro* and *in vivo* assays and has been shown to be a highly potent and selective 5-HT_3 receptor antagonist. It has been developed for the prevention of nausea and vomiting produced by cytotoxic drugs and radiation therapy, for which it is currently marketed.

FURTHER CLINICAL USES FOR 5-HT_3 RECEPTOR ANTAGONISTS?

Several potential clinical uses in addition to prevention of nausea and emesis have been proposed for 5-HT_3 receptor antagonists, including the treatment of anxiety, schizophrenia, cognitive disorders, drug addiction, pain, gastrointestinal motility disorders and pulmonary embolism. However, none of the currently available compounds have obtained regulatory approval for indications other than prevention of nausea and vomiting. Nevertheless, there is abundant evidence based on both preclinical and clinical studies that 5-HT_3 receptor antagonists have not yet realized their full therapeutic potential.

CONCLUSION

A long-term effort by many scientists to understand the physiology and pharmacology of 5-HT-mediated processes led to the realization that several subtypes of 5-HT receptor exist. To characterize them precisely, selective agonists and antagonists had to be synthesized. Potent selective competitive antagonists for one of these receptor subtypes, the 5-HT_3 receptor, were subsequently shown to be highly effective in preventing nausea and vomiting caused by cytotoxic chemotherapy or radiotherapy in cancer patients. The discovery of these compounds has thus not only contributed to a basic understanding of the actions of 5-HT on biological systems, but has shed light on the mechanisms involved in the control of nausea and vomiting and thus provided a considerable improvement in the clinical management of cancer.

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My thanks also go to Drs John Fozard and Rudolf Giger for reading the manuscript.

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Chapter 4

The clinical approach to chemotherapy-induced emesis

R. J. Gralla

INTRODUCTION

Improving the control of chemotherapy-induced emesis has been a major goal in the treatment of cancer. Most patients given chemotherapy now need not experience vomiting, and for those who have emesis, the severity of this side-effect has been greatly reduced. Although this represents a marked advance, complete control in all settings has not been achieved.

To evaluate needs for further improvement, it is necessary to assess the current degree of progress. This progress required a better understanding of the physiology of emesis, the identification of effective anti-emetics, and the use of established research methodology. With the new serotonin antagonists, several agents are now available either in clinical practice or in trials. The purpose of this paper is to outline advances and areas of concern, as summarized in Table 1, in the control of nausea and vomiting associated with chemotherapy.

PREVENTING EMESIS

Of the major emetic problems induced by chemotherapy (acute, delayed and anticipatory), the best studied and controlled is acute emesis. The last 15 years of investigation have improved complete control to a rate of at least 80% from a less than 1% rate before that time (1–3). Several aspects of treatment have led to this change.

The principles involved in the prevention of emesis include:

1) selection of the most active agents;
2) combination anti-emetic therapy of a 5-HT_3 receptor antagonist with a corticosteroid;
3) utilization of the most effective doses and schedules with minimization of side-effects.

Anti-emetic agents and doses

Metoclopramide (2, 3) provided the first serotonin-receptor antagonist (5-hydroxytryptamine type 3 or 5-HT_3) anti-emetic. In that metoclopramide also affects dopamine receptors, in high doses its efficacy is probably mediated by blocking

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Table 1. Progress and problems in controlling emesis in oncology.

Clinical situation	Comments
Acute emesis	
Complete control: cisplatin	⩾ 80% control with combination regimens
Complete control:other agents	⩽ 90% control with combination regimens
Consecutive day treatment	Poorer control; few well-conducted studies; problems of delayed and anticipatory emesis as well
High dose chemotherapy/transplant	Poorer control; few well-conducted studies; problem of radiotherapy-induced emesis in many cases
Radiation therapy	Overall, less of a problem than with chemotherapy; problem with consecutive day treatment; few well-controlled studies
Paediatrics	5-HT_3 antagonists effective; doses and schedules not well defined; few well-designed studies
Delayed emesis	
Complete control	Improvements needed; corticosteroids effective and corticosteroids + metoclopramide superior but regimens are cumbersome; role of 5-HT_3 antagonists unclear
Anticipatory emesis	
Prevention	Cornerstone of treatment; need to use the best regimens for acute and delayed emesis
Treatment	Some efficacy with behaviour therapy techniques
General considerations	
Side-effects	Low; easily managed with 5-HT_3 antagonists and with combination regimens
Convenience/flexibility	High; single-dosing regimens are best; regimens are easily applied to in-patient or out-patient settings; all-oral regimens likely to enhance convenience
Cost	Generally high; savings achievable by using effective lower dose regimens, single dosing, oral regimens

5-HT_3 receptors. With the development of the selective 5-HT_3 receptor antagonists, more specific treatments with fewer side-effects and greater convenience became the standard. Studies comparing metoclopramide regimens with different selective 5-HT_3 receptor antagonists revealed either similar or superior efficacy for the later agents (4). This efficacy, coupled with greater ease of use, made the 5-HT_3 receptor antagonists the treatment of choice in moderate to severe emesis.

Are there differences among the serotonin antagonists? In structure, potency per milligram, and pharmacokinetic profile, differences exist. In efficacy and side-effects of clinical importance, the agents appear to be remarkably similar. Several multicentre, prospective, random-assignment comparison trials have now been completed. These studies reveal equivalence between ondansetron and granisetron (5), and ondansetron and dolasetron (6) with identical efficacy using a variety of doses and schedules.

A low incidence of side-effects is shared by these drugs, as listed in Table 2 (4–6). Headache is the most frequent complaint (occurring in 15% to 30% of patients); however, this problem is usually minor and relieved without treatment or with such medications as paracetamol (acetaminophen). Gastrointestinal side-effects include diarrhoea, constipation, and elevation of transaminases. The latter effect is not of clinical significance, and as with diarrhoea or constipation, may be caused by the chemotherapy in many instances. The selective serotonin antagonists have anti-arrhythmic properties and several agents have been demonstrated to prolong the QTc and other electrocardiographic intervals (7). To date, no clinical problem related to this finding has emerged.

Older anti-emetic agents

In the past, many other agents have been used for chemotherapy-induced emesis. The phenothiazines, such as prochlorperazine, are commonly used in general medicine. High intravenous doses are better than the oral and intramuscular phenothiazines, however, orthostatic hypotension is a frequent toxicity. Other side-effects include sedation and dystonic effects (2).

Lorazepam has only modest anti-emetic effects, but with its anti-anxiety and amnestic properties it has high subjective acceptance. It is also effective in reducing akathisia associated with metoclopramide, and is useful in lessening dystonic

Table 2. Single-dose use of serotonin antagonist anti-emetics (4–6, 11–15, 25).

Agent	Recommended i.v. dose
Dolasetron	1.8 mg/kg
Granisetron	10 μg/kg
Ondansetron	8 mg or 0.15 mg/kg
Tropisetron	5 mg

Time of administration: immediately before chemotherapy

Side-effects: headache, elevation of liver enzymes, constipation

reactions. The major side-effect is sedation. Lorazepam is recommended as an addition to effective anti-emetics, primarily for its anti-anxiety effects, and not as a single agent (8).

Cannabinoids such as dronabinol (delta-9-tetrahydrocannabinol) have activity similar to oral phenothiazines, but markedly less than with metoclopramide or the 5-HT_3 receptor antagonists (9). Side-effects are greater than those of the phenothiazines or other anti-emetics and include sedation, dizziness, ataxia, orthostatic hypotension, dry mouth and dysphoria (1, 9). Although these agents have some anti-emetic properties, and other available agents have more activity and fewer side-effects, the role of cannabinoids in anti-emetic, treatment has not been well defined. Cannabinoids have great popularity in the lay press, independent of their tested properties. In addition, one well conducted (random assignment, double blind, cross-over) study compared oral tetrahydrocannabinol (THC) with inhaled marijuana. Both agents had similar, but low activity, with a trend toward patient preference for the THC (10).

Anti-emetic dosing schedules

Controversy persists concerning the ideal doses for the serotonin antagonists. The most practical and cost-effective approach in any setting is a single-dosing regimen. The drugs are not schedule-dependent, and single dosing regimens beginning immediately prior to cisplatin administration are very effective. The majority of reported studies indicate that single granisetron doses in the 1 mg range (10 μg/kg) and of ondansetron in the 8 mg to 0.15 mg/kg range, are more effective than lower doses and as effective as higher doses (Table 2 (11–15)).

Combination regimens with corticosteroids

Anti-emetic regimens combining a single dose of dexamethasone with either a serotonin antagonist or with metoclopramide are more effective than a single agent. This has been demonstrated in at least a dozen studies, with several anti-emetics, and for emesis induced by cisplatin and other chemotherapeutic agents (16–19). Little testing has examined different corticosteroid doses (16); still, a 20 mg dose of dexamethasone is highly effective, easy to administer, and inexpensive in all countries in which it is available generically.

Typically, anti-emetic efficacy with cisplatin will rise from the 40–50% range of complete control to 80% when dexamethasone is added to a 5-HT_3 receptor antagonist (16–20). With moderately emetogenic agents (cyclophosphamide, anthracyclines, carboplatin) the complete control rate rises to over 90% with the combination regimen, compared with either a 5-HT_3 receptor antagonist or dexamethasone alone (18). Recommended regimens are listed in Table 3.

After more than a decade of use in combination regimens, few negative factors have emerged with dexamethasone in this single-dose setting. Specifically, there has been no evidence of impaired therapeutic response from chemotherapeutic agents, and only minor side-effect considerations. Combination anti-emetic regimens with

Table 3. Combination anti-emetic regimens given once, immediately before cisplatin or moderately emetogenic chemotherapy (16–20, 30).

I.v. regimens	
Dexamethasone	20 mg over 5 minutes
+	
Ondansetron	8 mg or 0.15 mg/kg over 15 minutes
Dexamethasone	20 mg over 5 minutes
+	
Granisetron	1 mg or 10 μg/kg over 5 minutes
Oral regimen*	
Dexamethasone	20 mg
+	
Granisetron	1 mg (1.5 mg with high-dose cisplatin)

* Can be given as the injectable agents dissolved in 30 mg of water, or as tablets

corticosteroids should be considered treatments of choice for emesis caused by cisplatin or by agents of moderate potential to induce emesis.

Persistent problems

In several emetic situations, problems continue, as outlined in Table 1. One difficulty is that found with consecutive days of administration of chemotherapy. Regimens containing steroids may have improved efficacy, but further trials and approaches are needed. The phenomenon is complicated, in that it may involve elements of acute, delayed, and anticipatory emesis concurrently.

The serotonin antagonists, with their absence of dystonic side-effects, are particularly suited for use in children. It is noteworthy that although evidence of efficacy and safety in children is clear, few studies have been rigorously conducted. It is unfortunate that the paediatric oncology groups have not included well conducted anti-emetic trials as high priority studies to complement their chemotherapy protocols. Additionally, more rigorously conducted studies investigating radiation-induced emesis and the emesis associated with high-dose chemotherapy are needed.

Delayed and conditioned emesis begin after the administration of chemotherapy. Delayed emesis is often defined as that emesis beginning 24 hours after treatment (21). The potential of chemotherapeutic agents to cause delayed emesis parallels that seen with the acute problem. If patients receive treatment for acute emesis, but not for delayed, the delayed emesis incidence rate is 60–80% in patients treated with cisplatin, and 20–30% of those given carboplatin, cyclophosphamide or doxorubicin (18, 21, 22).

Treatment of delayed and anticipatory emesis differ from acute emesis. The key agents for delayed emesis are corticosteroids. Adding oral metoclopramide enhances

this activity (20, 22), but controversy continues concerning whether 5-HT_3 receptor antagonists are useful or indicated in the delayed emesis setting (4, 18, 21, 23–27). Some recent encouraging trials have started combination anti-emetics for delayed emesis at 16 hours after chemotherapy rather than at 24 hours (28), theorizing that delayed emesis begins in the 17–24 hour period based on the time of failure of control of acute emesis (4).

Conditioned or anticipatory emesis is the result of poor control of acute or delayed emesis. It is typically associated with anxiety prior to the next chemotherapy, followed by nausea or vomiting prior to, or during, chemotherapy administration. Although prevention of this problem with the most effective anti-emetics during each course of chemotherapy remains the best approach, treatment once the problem has occurred can be helpful. Behaviour therapy techniques with desensitization can be useful (29). The utility of anti-anxiety agents has not been well studied.

MAKING ANTI-EMESIS CONVENIENT, FLEXIBLE AND ECONOMICAL

Progress in anti-emetic research has indicated approaches that are universal in their application to clinical settings. In that single-dosing regimens are as effective as multiple doses, and with the ability to deliver these regimens over 5–20 minutes, several convenient combination regimens that are flexible enough for both outpatient and inpatient use are available (Table 3).

A recent phase II study demonstrated encouraging results with an all-oral single-dose combination regimen in 61 patients receiving high dose or moderate dose cisplatin ($\geqslant$ 100 mg/m^2 or $\geqslant$ 60 mg/m^2, respectively). Patients were given dexamethasone 20 mg orally plus granisetron at either 1 mg orally (moderate dose cisplatin) or 1.5 mg orally (high-dose cisplatin). The regimens were given 30 minutes before cisplatin, and 85% of patients had no emesis in the acute period (30). Additionally, over 70% of patients had complete control of delayed emesis with a regimen beginning 16 hours after cisplatin, as discussed above (28). This all-oral regimen for acute emesis could probably begin immediately before chemotherapy and provide the most convenient approach. The effectiveness of lower doses of anti-emetics, with the decreased need for nursing, drug administration time and pharmacy preparation, imply favourable cost savings (Table 1).

CONCLUSION

The future direction of anti-emetic research can take many different paths. Better understanding of the roles and interactions of neurotransmitter receptors in emesis will allow for different approaches to this problem. It appears unlikely that serotonin or dopamine pathways are the only ones involved in chemotherapy-induced emesis.

With 25% or more of patients not having complete control despite the use of active anti-emetics, investigation of anti-emetics with different mechanisms of action is a logical approach.

The considerable progress in anti-emetic therapy has been accomplished through complementary work conducted in several nations. The enhanced control of chemotherapy-induced emesis provides a model of how thoughtful research in supportive care can result in marked improvement for patients. Continued investigations are needed to solve the remaining problems in emesis.

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Chapter 5

How do toxic emetic stimuli cause 5-HT release in the gut and brain?

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INTRODUCTION

There is now abundant evidence to confirm the role of serotonin (5-HT), and in particular, 5-HT_3 receptors in the control of cisplatin-induced emesis (1). Emesis caused by cisplatin (and copper sulphate) is associated with an increase in the concentration of 5-HT in the intestinal mucosa (2, 3) and in the area postrema (4). The intestinal mucosa contains enterochromaffin (EC) cells, which synthesize and secrete approximately 90% of all 5-HT produced in the body. It is proposed that anticancer drugs cause 5-HT release from the EC cells and that the released 5-HT stimulates the 5-HT_3 receptors on the afferent vagal fibres, and results in depolarization. The vagus is the major afferent pathway involved in the detection of emetic stimuli (5). In animals, electrical stimulation of the abdominal vagal afferents is capable of inducing emesis (5), and abdominal vagotomy suppresses cisplatin-induced vomiting (3, 5).

How do toxic emetic stimuli cause an increase in 5-HT concentration and release in the gut and brain, and what is the role of this 5-HT? Because of inter-species variations in emetic mechanisms, ferrets were used throughout the experiments described in this Chapter. These constitute histopathological, biochemical and electrophysiological evaluation of cytotoxic drug-induced emesis.

STUDIES ON 5-HT IN THE ILEUM OF THE FERRET

Enterochromaffin cells synthesize and secrete 5-HT in the gut. Treatment of ferrets with cisplatin results in histological damage to the gut, which is maximal in the ileal mucosa (2). It has been reported previously that cisplatin, cyclophosphamide and copper sulphate produce a significant increase in ileal 5-HT levels in the ferret (2–4), and it has also been reported that 5-HT release from the isolated ileum of the guinea-pig and the cat is increased by cisplatin. We have measured the biosynthetic and metabolic enzyme activities of ileal 5-HT in ferrets in order to elucidate the mechanism whereby 5-HT release is increased (6). As shown in Figure 1, cisplatin- and cyclophosphamide- treated ferrets show significant increases in the activity of tryptophan hydroxylase (TPH), the rate limiting enzyme for the synthesis of 5-HT,

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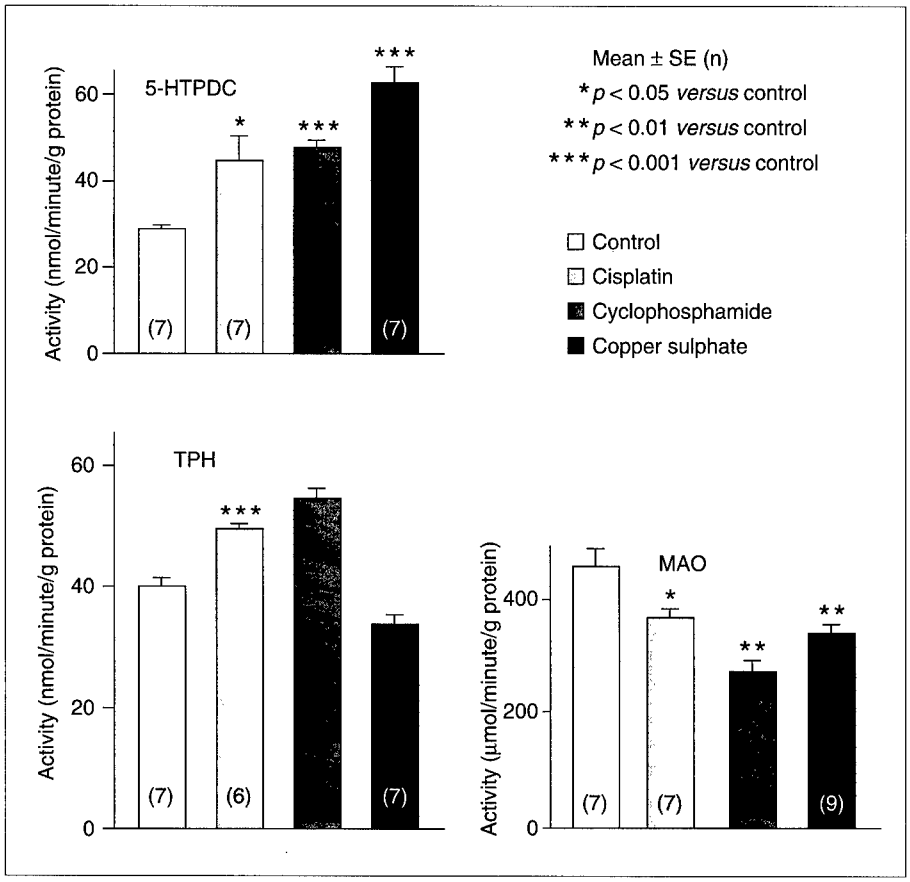

Figure 1. Effects of emetic agents on tryptophan hydroxylase (TPH), 5-hydroxytryptophan decarboxylase (5-HTPDC) and monoamine oxidase (MAO) activity in ferret ileum.

in the ileum, as compared with control animals. Aromatic L-amino acid decarboxylase (AADC) activity is also increased after the administration of these drugs. On the other hand, cytotoxic drugs induce a significant decrease in the activity of monoamine oxidase (MAO) in the ileum. Thus, cytotoxic drugs may activate ileal 5-HT biosynthesis and further enhance the concentration of 5-HT by reducing its degradation. However, cisplatin administered to ondansetron-pretreated ferrets induced no significant changes in TPH and MAO activity (6).

Using a modification of the method described by Milano, 5-HT release from isolated ferret ileum removed 60 minutes after cisplatin administration has been investigated (6). In this model, cisplatin (10 mg/kg, i.p.) produced a significant increase in cumulative 5-HT release that was approximately 2-fold greater than that of the control group. Pretreatment with ondansetron did not alter 5-HT release from the ileum.

Examination of the ileum of cisplatin-treated ferrets using electron microscopy

(Figure 2) demonstrates that 5-HT is stored in large electron-dense granules, which move towards the base of the EC cells and are released from the basal surface. We examined the EC cells 6 hours after cisplatin administration and found them empty of 5-HT. Mast cells were still intact 6 hours after cisplatin administration. This suggests that the source of 5-HT that is released during emesis is the EC cells.

VAGAL AFFERENT ACTIVITY IN THE FERRET

Afferent abdominal vagal activity was recorded from the peripheral cut end of the dorsal abdominal vagus nerve, using bipolar platinum–iridium wire electrodes. Analysis of nerve activity was performed after the conversion of raw data to standard pulses by a window discriminator that distinguished the discharge of afferent fibres from background noise (8).

Intravenous bolus injection of 5-HT and 2-methyl-5-HT, a 5-HT_3 receptor agonist, produced a dose-dependent increase in abdominal afferent vagus nerve activity in ferrets. N-3389 (9), a new 5-HT_3 receptor antagonist, significantly inhibited the 2-methyl-5-HT-induced increase in vagus nerve activity. As shown in Figure 3, cisplatin (10 mg/kg i.p.) also produced a significant increase in afferent abdominal vagal activity approximately 90 minutes after administration. The time-course of cisplatin-induced emesis in another group of ferrets paralleled the changes in the afferent vagus nerve activity. Ondansetron (0.1 mg/kg i.v.) and N-3389 (0.1 mg/kg i.p.) significantly inhibited the increase in vagus nerve activity induced by cisplatin (Figure 3). Administration of vehicle (physiological saline) did not mimic the afferent abdominal vagal activity. Although the afferent vagus nerves are considered to have 'polymodal' properties, the increase in the afferent vagal activity induced by cisplatin was significantly inhibited by 5-HT_3 receptor antagonism. Furthermore, we found that granisetron blocked the increase in afferent vagal activity induced by ouabain (9). Afferent vagus nerve activity increased immediately after copper sulphate administration (40 mg/kg p.o.) (Figure 3). Emetic episodes were also evoked immediately after copper sulphate administration in another group of ferrets. These findings demonstrate that the activation of vagal afferent activity may be relevant to cytotoxic drug- or copper sulphate-induced emesis in the ferret. This excitatory response may be mediated by the action of 5-HT on 5-HT_3 receptors located on the vagal afferent fibres.

SEROTONIN CONCENTRATIONS IN THE AREA POSTREMA OF THE FERRET

Receptor binding and autoradiographic studies of ferret tissues have shown that the area postrema contains high concentrations of 5-HT_3 receptors (1), and these receptors are likely to be important in the control of emesis (see Chapter 11).

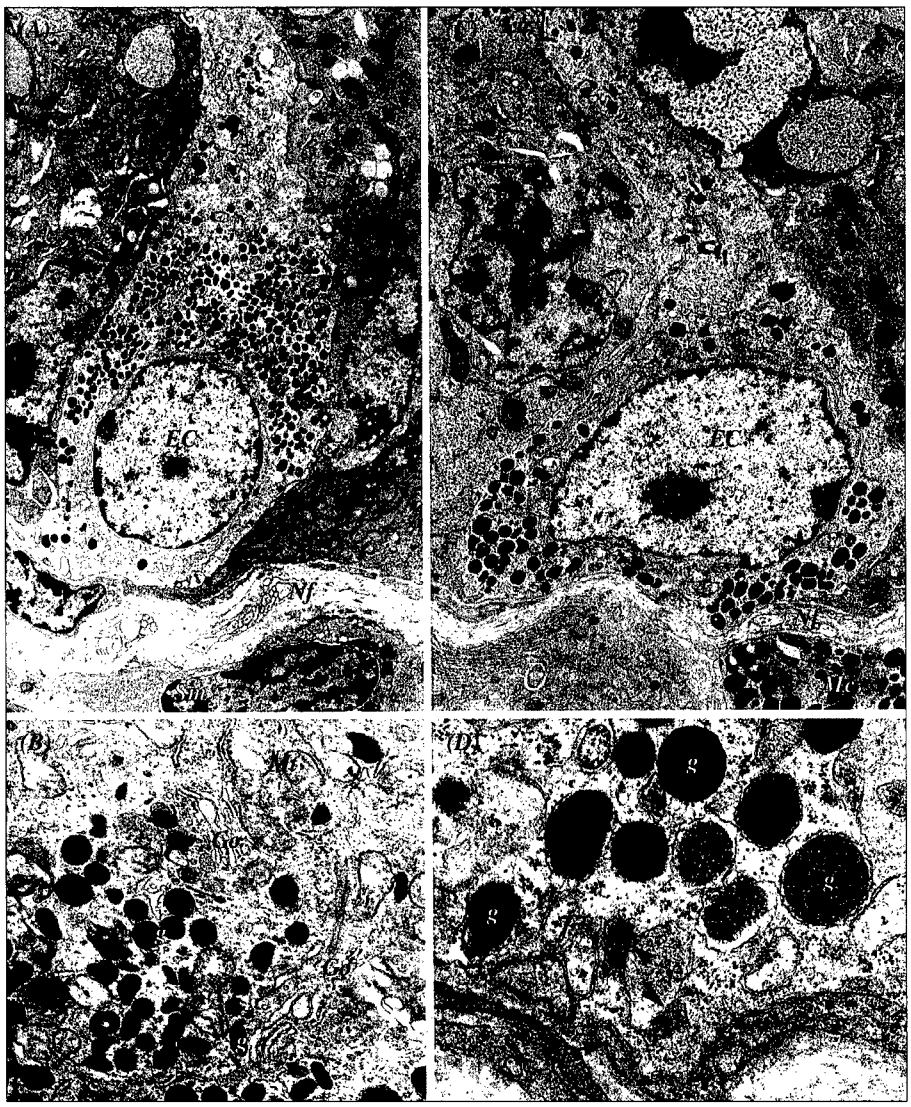

Figure 2. Electron microscopic view of enterochromaffin (EC) cells in ferret ileum.
A: Forming-phase of an EC cell. Many secretory granules are recognized in the supranuclear region (Golgi area).
B: Golgi area of a forming-phase EC cell. Secretory granules (g) are being formed in the Golgi apparatus.
C: Degranulating-phase of an EC cell. The basal granules are decreased in number.
D: Basal portion of a degranulating-phase EC cell. Secretory granules (arrowheads) are opening to the extracellular space (asterisk).
Bl, basal lamina; g, secretory granules; Go, Golgi apparatus; Lu, lumen of ileal crypt; Mc, mast cell; Mt, mitochondria; Nf, nerve fibres; Sm, smooth muscle.

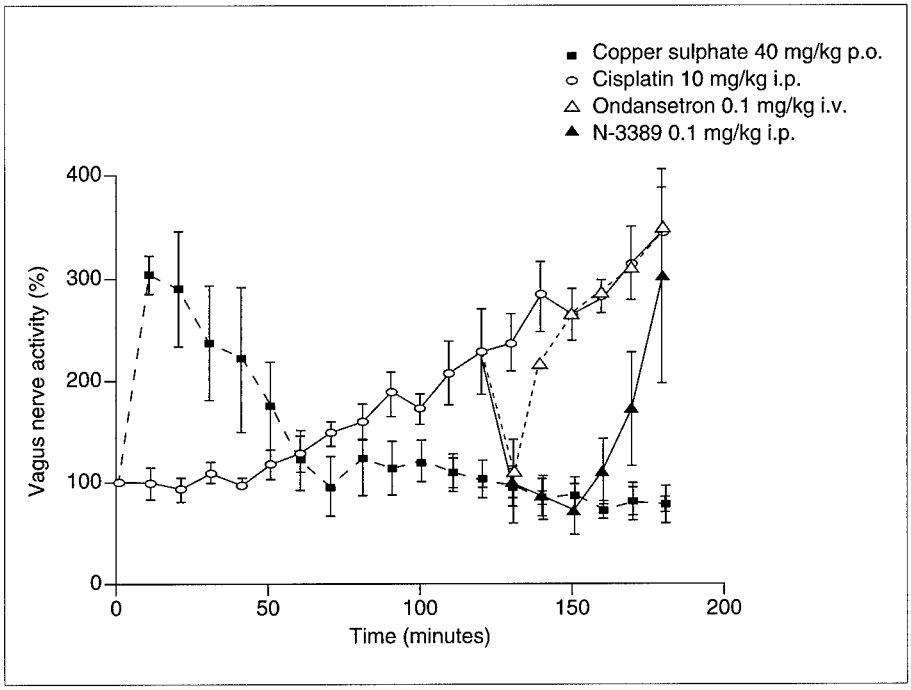

Figure 3. Effects of cisplatin and copper sulphate on the abdominal vagal afferent nerve activity in ferrets. Each point indicates the mean data of the afferents obtained from six ferrets.

In an attempt to clarify the mechanisms of emesis induced by cytotoxic drugs, we have measured the tissue concentrations of 5-HT in the ferret area postrema (4). As shown in Figure 4, the concentration of 5-HT in the area postrema of cisplatin- and cyclophosphamide-treated ferrets is significantly increased compared with those of saline-treated control animals. Pretreatment with ondansetron or abdominal vagotomy significantly inhibited this cytotoxic drug-induced increase in the 5-HT concentration of the area postrema (see Chapter 11).

Taken together, these results suggest that cytotoxic drugs induce emesis mainly through actions on the gastrointestinal tract. In ferrets, the area postrema contains a high concentration of 5-HT_3 receptors (1), it receives visceral afferent innervation from the gastrointestinal tract (5), and its 5-HT levels are increased and decreased by cytotoxic drugs and ondansetron, respectively (4). The area postrema, therefore, acts as a relay between the visceral afferents of the vagus and the vomiting centre (1). Cytotoxic drugs may cause 5-HT release from the EC cells of the intestinal mucosa to stimulate 5-HT receptors on the afferent vagal fibres. We have demonstrated that electrical stimulation of the abdominal vagal afferents induces an increase in the concentration of 5-HT in the area postrema.

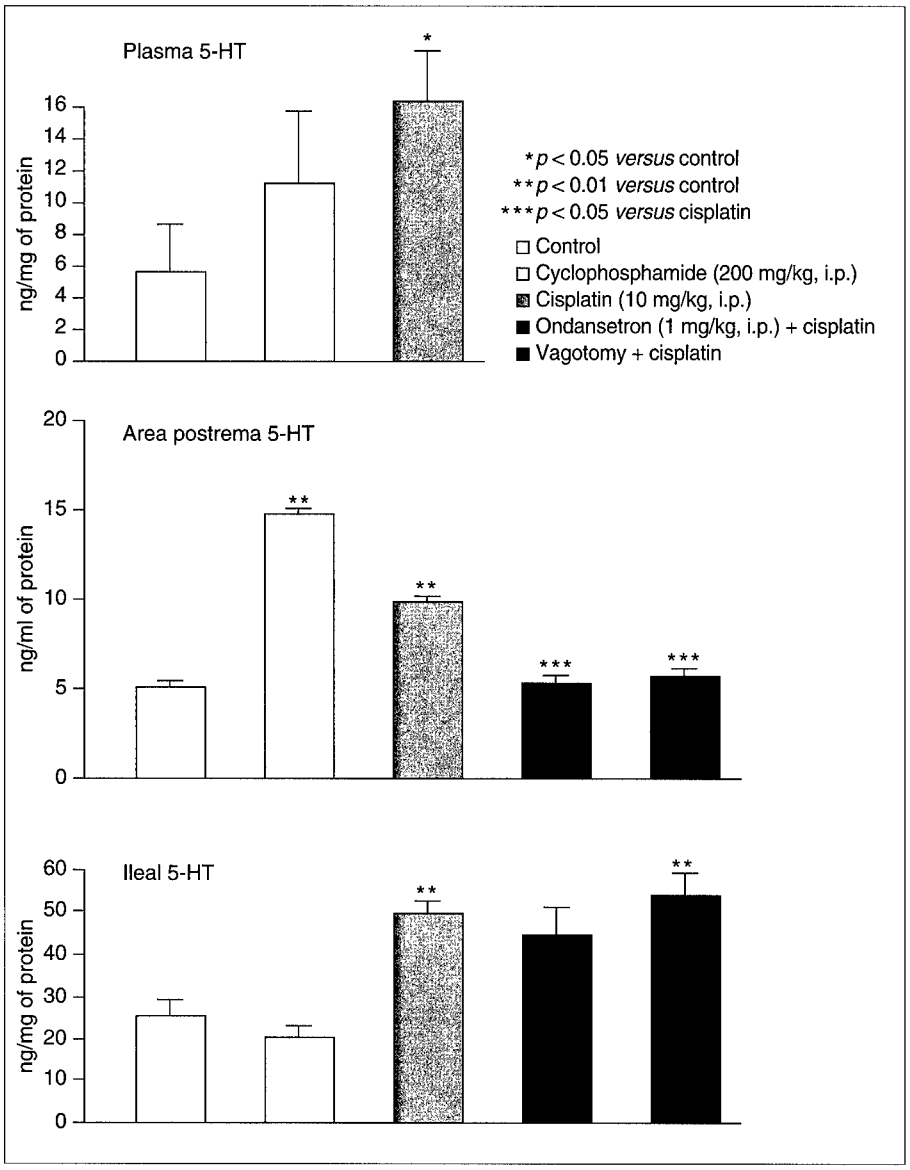

Figure 4. Effects of ondansetron and vagotomy on the increase in 5-HT concentration induced by cisplatin in the area postrema, ileum and plasma of ferrets.

Although platelets could release 5-HT during their passage through the area postrema, vagotomy clearly inhibited the increase of 5-HT in the area postrema. Finally, we have demonstrated that stimulation from afferent vagal fibres appears

to produce an increase in 5-HT concentrations in the area postrema. These findings suggest that this increase in 5-HT in the area postrema might trigger the emetic response induced by cytotoxic drugs in ferrets.

SEROTONIN AND COPPER SULPHATE-INDUCED EMESIS IN THE FERRET

Copper sulphate is generally considered to be a peripherally acting agent causing gastrointestinal irritation that stimulates the emetic reflex through unspecified transmitter mechanisms. As 5-HT_3 receptor antagonists can inhibit emesis caused by chemotherapeutic agents, the role of 5-HT in emesis induced by copper sulphate has been investigated (3). Copper sulphate produced a dose-related increase in emesis in ferrets treated with 20 mg/kg (3/6 animals), and 40 mg/kg (6/6 animals). Ondansetron reduced the number of retches and vomits induced by copper sulphate, 40 mg/kg, by a half. The copper sulphate-treated animals showed a significant increase in ileal mucosal levels and area postrema levels of 5-HT. Copper sulphate-treated ferrets had significant increases in AADC activity, and reductions in MAO activity, in the ileum. These increased levels of 5-HT might play a triggering role in vomiting induced by copper sulphate. The precise mechanism of how copper sulphate releases 5-HT from EC cells remains unknown. Torii *et al.* (10) speculated that cisplatin releases 5-HT by generating free radicals. Copper sulphate is also capable of accelerating the production of free radicals. Further study is necessary to investigate the relationship between copper sulphate-induced emesis and free radical formation.

DISCUSSION

The concentration of 5-HT in the ileum of animals treated with copper sulphate is significantly increased compared with that of the physiological saline-treated control ferrets (3). A similar situation occurs after cisplatin and copper sulphate administration (2). Treatment with cisplatin and copper sulphate results in an increase in the activity of abdominal vagal afferent fibres in anaesthetized ferrets. This is in agreement with lesioning studies, which have shown the emesis induced by cytotoxic drugs and copper sulphate is reduced by vagotomy (5). Cisplatin also produces a release of 5-HT from an isolated ileal mucosa preparation in ferrets (6). This 5-HT release from the ferret ileum occurs 100 minutes after cisplatin administration (6), a time-course that coincides well with behavioural changes and the increase in afferent nerve activity. In other words, the concentration of 5-HT in the ileum increases synchronously with vomiting. The 5-HT released activates 5-HT_3 receptors at the afferent vagal nerve endings near the basal membrane of the EC cells and it is this stimulation of 5-HT_3 receptors that induces EC of the vagal

fibres, and evokes the vomiting response (5). Our studies confirm the significant role of the intestinal afferent vagal nerve activity in the control of emesis.

Cyclophosphamide and cisplatin result in significant increases in the concentration of 5-HT in the ferret area postrema compared with saline-treated animals, and these increases are significantly inhibited by ondansetron or abdominal vagotomy (4). This suggests that increases in 5-HT concentration in the area postrema occur as a result of vagal afferent activity rather than a direct action of cytotoxic drugs at this level.

The present findings suggest the following hypothesis. Cytotoxic drugs have an initial action within the gut that results in histological changes, activation of the biosynthesis of 5-HT and an increase in the concentration of 5-HT in the gastrointestinal tract. 5-HT is released from the EC cells of the intestinal mucosa, which in turn stimulates 5-HT_3 receptors on vagal afferent fibres. This stimulation of vagal afferent fibres results in an increase in 5-HT in the area postrema, and leads to emesis.

ACKNOWLEDGMENTS

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Chapter 6

The role of free radicals and nitric oxide in the induction of emesis

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INTRODUCTION

We have been working on a non-rodent animal model, *Suncus murinus*, the house musk shrew, to investigate the mechanism of cisplatin-induced emesis. As has been reported by a number of researchers, the involvement of 5-HT and 5-HT_3 receptors in emesis is well established. However, very little is known about why 5-HT_3 antagonists are so effective against cisplatin-induced emesis. It is believed that cisplatin releases 5-HT from the enterochromaffin cells, and that 5-HT_3 receptor antagonists compete with this 5-HT for receptor sites on vagal afferent terminals. However, the mechanism(s) by which cisplatin causes 5-HT release are unknown. At a meeting in Marseille in 1992, we first proposed that oxygen free radicals might be involved, and in this presentation we provide further evidence for the involvement of free radicals in cisplatin-induced release of 5-HT.

SUNCUS MURINUS

Suncus murinus is a small mammal that belongs to the order Insectivora. The insectivores are considered to be the most primitive and the oldest placental mammals. We introduced this animal model for the study of emesis about 8 years ago (1–3) and have subsequently worked to define its emetic characteristics. Several colonies are already established in Europe. We have also shown that *Suncus murinus* is not the only insectivore that can vomit. *Sorex unguiculatus* can also vomit (4). The body weight of adult *Sorex unguiculatus* is only 10 g and, to date, this animal is the smallest known mammal that can vomit. Emesis in *Suncus murinus* consists of several very rapid retches (with a frequency as high as 4 Hz) and subsequent vomiting. The initial vomit is always accompanied by an expulsion of gastric and sometimes bile-stained contents. Because of the rapidity with which individual retches and vomits occur, we have defined the emetic response of *Suncus murinus* in terms of 'vomiting episodes' each of which incorporates several retches and vomits but occurs as a well defined bout of emetic activity.

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THE SITE OF ACTION OF CISPLATIN

It is likely that cisplatin and 5-HT_3 receptor antagonists work peripherally in *Suncus* for the following reasons:

1) cisplatin-induced emesis is completely blocked by surgical abdominal vagotomy (5);
2) intracerebroventricular injection of cisplatin does not cause emesis (Matsuki *et al.* unpublished);
3) tropisetron, a 5-HT_3 receptor antagonist, specifically blocks cisplatin-induced emesis when injected systemically, but not intracerebroventricularly (Matsuki *et al.* unpublished).

These results suggest that central 5-HT_3 receptors are not essential to cisplatin-induced emesis in *Suncus*. However, it is still possible that central 5-HT_3 receptors have a modulatory role. Recent histochemical studies in our laboratory analysing the expression of c-Fos protein, have revealed that neural activation in both the nucleus tractus solitarius, and dorsal motor nucleus of the vagus occurs after injection of cisplatin, and this can be reduced by pretreatment with tropisetron, and to a lesser extent by vagotomy. Therefore, as the site of action of cisplatin in *Suncus* appears to be peripheral (see above), stimulation of central 5-HT_3 receptors is either independent of cisplatin-induced emesis or insufficient to cause emesis.

IS CISPLATIN ITSELF EMETOGENIC?

The latency to the onset of vomiting after intraperitoneal injection of cisplatin is nearly 1 hour and does not become shorter when injected intravenously. One interpretation of this is that cisplatin may have been converted to one or more metabolites that causes vomiting. The platinum complex is not a stable compound and it binds readily to serum proteins. This makes it very difficult to analyse metabolites of cisplatin using a conventional HPLC system. Several active metabolites are proposed to be responsible for the cytotoxic effects of cisplatin. We have focused on one of them, *cis*-diaquodiammineplatinum (II) (DAP) the cytotoxicity of which is comparable to that of the original compound (5). When DAP is injected intraperitoneally, the latency of emesis is decreased to 20 minutes, compared with 50 minutes for cisplatin. DAP-induced emesis is completely blocked by a 5-HT_3 receptor antagonist or by abdominal vagotomy. Although these lines of evidence are circumstantial, it is possible that cisplatin is converted to active metabolite(s), such as DAP, which may be involved in the emetic response.

INVOLVEMENT OF 5-HT IN CISPLATIN-INDUCED EMESIS

Subcutaneous, intraperitoneal, or intravenous injection of 5-HT causes emesis in *Suncus* with extremely short latencies of 20–50 seconds (6). 5-HT-induced emesis is completely blocked by surgical abdominal vagotomy or by a 5-HT_3 receptor antagonist. 2-methyl 5-HT, a selective 5-HT_3 receptor agonist, causes emesis that is also blocked by a 5-HT_3 receptor antagonist. Intraperitoneal injection of cisplatin or 2-methyl-5-HT increases discharges in vagal afferents (Matsuki *et al.* unpublished observations). The serum concentration of 5-HT and its metabolite, 5-HIAA, were increased 60 minutes after the administration of cisplatin when animals vomited vigorously (Torii *et al.*, unpublished). The 5-HT content of the intestine was slightly decreased. Four days' treatment with *p*-chlorophenylalanine, which decreased the intestinal content of 5-HT by 80%, inhibited cisplatin-induced emesis (Torii *et al.*, unpublished observation). Taken together, these results suggest that 5-HT is involved in, or mediates, cisplatin-induced emesis. Recently, we have obtained more direct evidence that cisplatin releases 5-HT from the isolated and perfused intestine (Matsuki *et al.*, unpublished observation).

X-IRRADIATION-INDUCED EMESIS

We have characterized X-ray-induced emesis in *Suncus murinus* (7), and have shown that there are great similarities between the emetic responses caused by cisplatin and X-rays, namely that:

1) abdominal but not head irradiation causes emesis;
2) it is blocked by a 5-HT_3 receptor antagonist;
3) it is blocked by surgical abdominal vagotomy.

INVOLVEMENT OF FREE RADICALS

The above mentioned results suggest that cisplatin is converted to one or more active metabolites that release 5-HT from the intestine, probably from the enterochromaffin cells, which then stimulates 5-HT_3 receptors located on vagal afferents. However, it is still not known how cisplatin or its active metabolites releases 5-HT. The similarity of cisplatin-induced emesis and irradiation-induced emesis prompted us to postulate that free radicals are common mediators of the response, as it is well known that radiation produces free radicals, which can have a variety of effects on cellular function.

First of all we measured lipid peroxide activity in various organs. The content of lipid peroxides was increased significantly after the injection of cisplatin in a

number of tissues including the intestine, suggesting that production of oxygen free radicals was occurring (8). If free radicals are responsible for cisplatin-induced emesis, antioxidants and free-radical scavengers should prevent it. However, results using superoxide dismutase (SOD) and catalase were disappointing. Neither pre- nor post-treatment with polyethylene glycol-conjugated SOD or catalase affected emesis. However, these enzymes cannot penetrate cell membranes, and if free radicals are produced in the enterochromaffin cells that release 5-HT, the results are not surprising. When we used a membrane permeable antioxidant, N-(2-mercaptopropionyl) glycine (MPG), it completely prevented the emesis. However, MPG did not affect 5-HT-induced emesis. Transition metals, such as iron catalyse the production of oxygen free radicals. Consistent with this we found that desferrioxamine, an iron chelator, attenuated cisplatin-induced emesis whereas ferric chloride exaggerated emesis (9). Furthermore, pyrogallol, a generator of hydroxy radicals, also caused emesis with characteristics similar to those induced by cisplatin, that is, it was blocked by surgical abdominal vagotomy, a 5-HT_3 receptor antagonist and MPG (10). As mentioned above, cisplatin causes 5-HT release from the isolated intestine. This release was completely prevented in the presence of acetyl cysteine, an antioxidant (Matsuki *et al.*, unpublished observation). These results provide strong evidence to support the idea that oxygen free radicals are involved in or mediate cisplatin-induced emesis (Table 1). This is not specific to *Suncus murinus*. Preliminary studies in the ferret have shown that pyrogallol can induce an emetic response that is blocked by a 5-HT_3 receptor antagonist, and is markedly reduced by surgical abdominal vagotomy combined with section of the greater splanchnic nerves (Andrews, Woods, and Matsuki, unpublished observations).

Table 2 summarizes the characteristics of emesis caused by various emetics. Interestingly, the latencies to emesis for DAP, X-ray irradiation and pyrogallol are all about 20 minutes. Therefore, it may take about 20 minutes to cause emesis after production of oxygen free radicals. Free radicals probably damage the membrane of vesicles containing 5-HT. However, further studies are necessary to clarify the precise mechanisms.

Table 1. Evidence that indicates the involvement of oxygen free radicals in cisplatin-induced emesis.

1	Cisplatin increases lipid peroxides in various organs including the intestine
2	Cisplatin-induced emesis is blocked by antioxidants
3	Cisplatin-induced emesis is attenuated by an iron chelator
4	Cisplatin-induced emesis is exaggerated by ferric ions
5	Pyrogallol, a generator of free radicals, and X-irradiation cause emesis with characteristics similar to that caused by cisplatin
6	Cisplatin-induced release of serotonin is blocked by an antioxidant

See text for details and references.

Table 2. Comparison of cisplatin-, DAP-, X-radiation-, 5HT- and pyrogallol-induced emesis in *Suncus murinus*.

Stimulus	Latency (minutes)	5-HT_3 receptor antagonist	Vagotomy	Antioxidant
Cisplatin	50	+	+	+
DAP	18	+	+	?
X-radiation	20	+	+	?
5-HT	< 1	+	+	–
Pyrogallol	19	+	+	+

+ = Blockade; – = No blockade; ? = Not tested. See text for references.

NITRIC OXIDE IS NOT A CANDIDATE

Nitric oxide (NO) has been shown to be an important mediator in a wide variety of physiological functions, including endothelium-mediated vascular relaxation, long-term potentiation and macrophage cytotoxicity. NO is a short-lived radical, synthesized from L-arginine by NO synthetase, and drugs that compete with L-arginine as substrates are used to block the synthesis of NO. Conversely, administration of L-arginine will increase the synthesis of NO.

It is conceivable that NO is produced in the enterochromaffin cells and is directly involved in the release of 5-HT after the administration of cisplatin. We investigated this possibility in *Suncus murinus*. Pretreatment of animals with N-nitro-L-arginine did not affect the number of vomiting animals nor the latency for emesis, but did decrease the total number of episodes (Table 3). However, doses as high as 256 mg/kg were necessary to obtain a significant effect. L-Arginine did not modulate cisplatin-induced emesis (Table 4). Administration of sodium nitroprusside or sodium azide, which releases NO directly, caused dose-dependent emesis (Table 5). However, nitroprusside-induced emesis was not prevented by the 5-HT_3 receptor antagonist tropisetron, or by the antioxidant MPG (Table 6). These results suggest that NO itself may be emetogenic but it is not likely to be the free radical responsible for the mediation of cisplatin-induced emesis.

Table 3. Effects of N-nitro-L-arginine on cisplatin-induced emesis.

	No. vomiting/ no. tested	Latency (minutes)	No. of vomiting episodes
Saline	8/8	43.0 ± 3.0	16.4 ± 2.7
N-nitro-L-arginine (256 mg/kg)	8/8	52.5 ± 4.2	8.2 ± 0.9*

Values represent mean ± SEM
N-nitro-L-arginine was administered 20 minutes before the injection of cisplatin (20 mg/kg i.p.)
*Denotes significantly different from saline by Student's *t*-test ($p < 0.05$)

Table 4. Effects of L-arginine on cisplatin-induced emesis.

	No. vomiting/ no. tested	Latency (minutes)	No. of vomiting episodes
Saline	3/3	39 ± 7	15 ± 3
L-arginine (256 mg/kg)	3/3	40 ± 5	14 ± 3

Values represent mean ± SEM
L-arginine was administered 30 minutes before the injection of cisplatin (20 mg/kg, i.p.)

Table 5. Emetic effects of nitric oxide-releasing drugs in *Suncus murinus.*

Dose (mg/kg)	No. vomiting/ no. tested	Latency (minutes)	No. of vomiting episodes
Sodium nitroprusside			
2	0/2	–	–
4	2/5	3, 3	4, 3
8	5/5	11.8 ± 1.9	2.4 ± 0.2
16	1/1	12	1
Sodium azide			
2.5	0/4	–	–
5	2/5	2, 5	18, 2
10	4/4	1.9 ± 0.1	7.3 ± 0.9
40	1/1	1	8
Hydroxylamine			
8	0/2	–	–
40	0/1	–	–
80	1/2	2	16
Sodium nitrite			
5	0/1	–	–
10	0/1	–	–
20	0/1	–	–
40	1/1	52	1
80	1/1	15	13

Table 6. Effects of tropisetron on sodium nitroprusside-induced emesis.

Pretreatment	Dose	No. vomiting/ no. tested	Latency (minutes)	No. of vomiting episodes
Saline	0	8/8	3.9 ± 1.0	12.9 ± 1.5
Tropisetron (μg/kg)	400	2/3	2.5, 4.1	11, 11
	800	4/4	3.3 ± 0.3	9.3 ± 2.1
MPG (mg/kg)	200	2/4	2.3,3.9	7,10
	400	4/4	4.1 ± 0.3	6.3 ± 2.0*

Values represent mean ± SEM. Sodium nitroprusside: 8 mg/kg, s.c.
MPG, N-(2-mercaptopropionyl) glycine
*Significantly different from saline group by Duncan's test ($p < 0.05$)

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Chapter 7

The pharmacology of 5-HT release from enterochromaffin cells

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INTRODUCTION

Enterochromaffin (EC) cells are a serotonin-rich population of neuroepithelial cells that are dispersed all over the intestinal mucosa (1, 2). Although serotonergic neurones are also present in the intestine (3), it should be emphasized that more than 95% of the intestinal 5-HT is present in EC cells. During the last decade numerous studies, particularly from our own laboratories, have revealed a complex pattern of regulatory mechanisms involved in the control of 5-HT secretion from the ECs. In this chapter we shall summarize the current knowledge about these control mechanisms (more comprehensively surveyed in (4–6)), paying particular reference to those areas that may be relevant in the initiation of emetic reflexes.

5-HT SYNTHESIS AND STORAGE

Isolated EC cells can accumulate 5-HT through a specific, imipramine-sensitive 5-HT uptake mechanism, and contain the enzymes to synthesize 5-HT from the amino acid tryptophan (i.e. tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase) (5). EC cells contain large, electron-dense secretory granules, in which 5-HT is stored together with a variety of different peptides. These secretory granules are concentrated at the base of the EC cells, suggesting that release at the basolateral membrane (i.e. the interstitial side) may be most important (see Chapter 5). Indeed, experiments in which the directional release of 5-HT has been studied in Ussing chambers have shown that a stimulus-dependent secretion of 5-HT occurs only from the interstitial side, and not from the mucosal surface (5).

5-HT RELEASE FROM ENTEROCHROMAFFIN CELLS

Until recently, an isolated cell preparation of EC cells was not available (5), so most of the studies on 5-HT secretion from these cells have been performed on isolated intestinal segments with the mucosa intact. 5-HT release from this preparation appears to reflect 5-HT secretion from EC cells, as 5-HT in the intestine is almost exclusively confined to these cells and hardly any 5-HT release is detectable in

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mucosa-free preparations (4, 6). In isolated intestinal segments, the autonomic nervous system is still capable of functioning, and drugs might affect 5-HT release indirectly through modulation of neurotransmitter release. Direct drug effects on EC cells in these preparations can, however, be demonstrated in the presence of the neurotoxin tetrodotoxin (TTx), that is, after blockade of the neuronal input to the EC cells.

General characteristics of 5-HT release

5-HT release from EC cells, like any neurosecretory process, may occur through exocytosis, and appears to be triggered by a rise in intracellular calcium, either by calcium influx through voltage-regulated channels or by receptor-mediated liberation of calcium from intracellular stores. Pharmacological experiments have indicated that the EC cells are endowed with multiple calcium channels, but those that have characteristics of L-type channels appear to be most important. On the other hand, fast sodium-dependent, 'neuronal type' action potentials appear not to be crucially involved in the stimulus-secretion coupling of EC cells (6).

Receptor mediated control of 5-HT release

Cholinergic receptors

In all species studied (guinea-pig, pig, dog and rabbit) muscarinic receptors mediate a facilitation of 5-HT release from EC cells through receptors located directly on the EC cells. This is indicated by experiments that show that muscarinic receptor agonists stimulate the release of 5-HT in the presence of TTx in all species. However, in some species (pig and guinea-pig) muscarinic receptor agonists inhibit 5-HT release in the absence of TTx, indicating that in these species, activation of neuronal muscarinic receptors can facilitate the release of inhibitory neurotransmitters. The muscarinic receptors on the EC cells belong to the M_3 subtype and appear to activate 5-HT secretion by liberating calcium from intracellular stores (5, 7), whereas the muscarinic receptors that mediate activation of the inhibitory neurones are of the M_1 subtype (4, 5).

Stimulatory nicotinic receptors on EC cells can only be demonstrated in the guinea-pig and porcine intestine. In the rabbit and canine intestine, stimulatory effects of nicotine on 5-HT release are mediated indirectly by activation of ganglionic nicotinic receptors on cholinergic neurones, and acetylcholine released in the vicinity of the EC cells has been shown to stimulate 5-HT secretion via muscarinic receptors (5, 6).

Adrenoceptors and role of cAMP

5-HT release from EC cells is facilitated by β adrenoceptors, localized directly on the EC cells, and this effect may involve stimulation of adenylyl cyclase, as inhibition of phosphodiesterase results in a potentiation of the stimulatory effect of isoprenaline. That an elevation of intracellular cAMP enhances 5-HT secretion is

further indicated by the stimulatory effects of the stable cAMP analogue 8-bromo-cAMP, and of forskolin, which directly stimulates adenylyl cyclase. Likewise, cholera toxin, which is known to cause a prolonged activation of adenylyl cyclase, also enhances 5-HT release (4, 5). The EC cells are, in addition, endowed with inhibitory α_2-adrenoceptors, but their signal transduction pathway in the EC cells is unknown, although α_2-adrenoceptors are often linked to inhibition of adenylyl cyclase (4, 5).

Histamine receptors

Histamine has been shown to inhibit 5-HT secretion from porcine small intestine by direct effects on EC cells. There are three major classes of histamine receptors, H_1, H_2 and H_3 receptors, and detailed pharmacological experiments have shown that the histamine receptors on the EC cells belong to the H_3 subtype (4, 5).

GABA and benzodiazepine receptors

There are two major classes of GABA receptor, $GABA_A$ and $GABA_B$, and both appear to play a role in the control of 5-HT release from the EC cells. The $GABA_A$ receptor is a chloride channel, and in most excitable cells the opening of this chloride channel results in a reduction of excitability. Similar mechanisms may occur in EC cells, where $GABA_A$ receptors mediate an inhibitory effect on 5-HT release. Further pharmacological studies with the benzodiazepine receptor agonist, midazolam, and the antagonist, flumazenil, indicate that the $GABA_A$ receptors on EC cells, like neuronal $GABA_A$ receptors, are modulated by benzodiazepine receptors. In addition, the EC cells are also endowed with inhibitory $GABA_B$ receptors, as baclofen, in the presence of TTx, reduces the release of 5-HT in a stereospecific manner (4, 5).

Purinoceptors

Adenosine and ATP inhibit 5-HT release from the porcine small intestine by a direct action on the EC cells, and different purinoceptors are involved in these effects. Thus, the effect of adenosine, but not that of ATP, is blocked by the A_2 receptor-selective antagonist CGS 15943A. The effect of ATP is inhibited by the P_{2y} receptor antagonist cibacron blue, and mimicked by the P_{2y} receptor agonist α–methyl-thio-ATP. P_{2y} receptors have recently been subdivided into $P_{2y\alpha}$ and $P_{2y\beta}$ subtypes, and the $P_{2y\alpha}$ subtype has been suggested to be a ligand-controlled cation channel (8), which can be blocked by d-tubocurarine. In the above experiments, d-tubocurarine prevented the inhibitory effect of ATP, suggesting the involvement of a $P_{2y\alpha}$ subtype. Moreover, apamin, a toxin that blocks calcium-activated potassium channels (9), also abolishes the inhibitory effect of ATP. Therefore, it is concluded that calcium influx through ATP-gated channels may activate potassium channels resulting in hyper-polarization of the EC cells and inhibition of 5-HT secretion. Interestingly, this putative calcium influx through ATP-gated channels does not activate 5-HT secretion, suggesting that a rise of intracellular calcium in different cellular compartments of the

EC cells can result in opposing functional responses. The polarity of these cells with the secretory active zone at the basolateral side was discussed above.

Peptide receptors

The EC cells are also endowed with a number of peptide receptors that mediate inhibition of 5-HT release. Somatostatin, and its stable analogue octreotide, inhibit 5-HT release from the rabbit small intestine evoked by a variety of stimuli. Octreotide potently inhibits release of 5-HT evoked by high potassium or forskolin, and is less effective when 5-HT release is evoked by muscarinic receptor activation. Thus, it appears that somatostatin receptors on EC cells affect multiple processes in the chain of events of stimulus–secretion coupling. Inhibitory effects of somatostatin on the calcium channels of the EC cells (high-potassium-evoked release), and on adenylyl cyclase (forskolin-evoked release) appear to be of great significance, whereas effects on 5-HT release triggered by liberation of intracellular calcium appears to be less important (7).

5-HT release from the guinea-pig small intestine is inhibited by vasoactive intestinal polypeptide (VIP) through a direct action on the EC cells. VIP and the related peptides PACAP-(127) and PACAP-(138) (pituitary adenylate cyclase activating peptide) also inhibit 5-HT release from the porcine small intestine (Schwörer and Racké, unpublished observations) and evidence has been obtained that VIP and PACAP may act through a common receptor, the PACAP-2 receptor.

5-HT autoreceptors

5-HT release from EC cells appears to be controlled by 5-HT autoreceptors, and evidence has been obtained for stimulatory 5-HT_3 and inhibitory 5-HT_4 receptors (10). Thus, 2-methyl-5-HT enhances 5-HT release from the guinea-pig small intestine in the presence of TTx, and this effect is inhibited by the antagonist tropisetron, in 5-HT_3 receptor-selective concentrations. Moreover, several 5-HT_3 receptor-selective antagonists reduce 5-HT release in this preparation, indicating that these 5-HT_3 receptors may indeed be autoreceptors, that is, a target for endogenous 5-HT released from the EC cells. However, the 5-HT_3 receptors on on these cells appear to be pharmacologically different from those on myenteric neurones (10). Furthermore, several 5-HT_4 receptor agonists inhibit 5-HT release in the presence of TTx. Finally, that these 5-HT_4 receptors on the EC cells may also be a target for endogenous 5-HT is suggested by the observation that tropisetron in higher concentrations (also blocking 5-HT_4 receptors), increases 5-HT release above basal levels. Thus, under the resting *in vitro* conditions the net effect of autoreceptor activation (5-HT_3 receptor facilitation and 5-HT_4 receptor inhibition) appears to be inhibition of 5-HT release.

Effects of cisplatin

Cisplatin is a cytotoxic drug used in anti-neoplastic chemotherapy. One of the most important acute undesirable effects is severe emesis that can be treated effectively by 5-HT_3 receptor antagonists (11). In addition, there is evidence that this emesis might

be triggered by an activation of intestinal vagal afferents, and that an enhancement of intestinal 5-HT release could be involved ((12); see also Chapter 5). Therefore, we have studied the effect of cisplatin on 5-HT release from isolated intestinal segments. Cisplatin caused a marked 5-HT release from the guinea-pig small intestine and the maximum effect of cisplatin was seen at a concentration of 3 μM, which corresponds well to the concentrations of cisplatin observed in patients during chemotherapy (13). Furthermore, it was shown that this effect of cisplatin was not caused by severe damage to the EC cells. On the contrary, this effect of cisplatin was abolished in the presence of 5-HT_3 receptor antagonists, TTx, hexamethonium or scopolamine, indicating that a cascade of neuronal events, involving cholinergic neurones and the stimulation of 5-HT_3 receptors is involved (13). In accordance with these observations it was shown on cultured dorsal root ganglion neurones that cisplatin can enhance neuronal excitability (14). Finally, 3 μM cisplatin also enhanced 5-HT release from isolated porcine small intestine, and this effect was again blocked by the 5-HT_3 receptor antagonist tropisetron (Schwörer and Racké, unpublished).

CONCLUSION

A complex pattern of receptor-mediated mechanisms has been shown to modulate 5-HT release from the EC cells, and involves different stimulatory receptors (β adrenoreceptors, muscarinic and nicotinic cholinergic receptors, and 5-HT_3 receptors), and inhibitory receptors (α_2-adrenoreceptors, histamine H_3, $GABA_A$ and $GABA_B$ receptors, A_2 and $P_{2\gamma\alpha}$ purine receptors, 5-HT_4, somatostatin, and VIP receptors (PACAP-2) receptors). Moreover, 5-HT release from EC cells is also stimulated by enterotoxins, such as cholera toxin, or by cytotoxic drugs used in cancer chemotherapy, such as the highly emetic agent, cisplatin. Enterochromaffin cells appear to represent a well-differentiated perceptive system of the intestinal mucosa, integrating numerous neuronal, humoral, paracrine and toxic inputs to the intestine.

ACKNOWLEDGEMENTS

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Chapter 8

The vagal afferent response to 5-HT and cisplatin

D. Grundy and K. Hillsley

INTRODUCTION

Since Paintal's demonstration in the 1950s that vagal afferent fibres supplying the gastrointestinal tract were stimulated by phenyl biguanide (1), there has been considerable interest in the role of serotonin (5-HT) in the transduction of afferent signals from the gut lumen. This is especially relevant as more than 90% of the 5-HT in the body is stored within the enterochromaffin cells of the gastrointestinal mucosa. These cells, together with other entero-endocrine cells in the mucosa, have a morphology that is consistent with a paracrine 'sensory' role (2). Thus, the apical membrane has specialized microvilli, which are proposed to 'sample' luminal contents, and 5-HT and peptides such as CCK are released across the basolateral membrane where afferent nerve terminals in the lamina propria are in close proximity. Some of these terminals are of neurones that are intrinsic to the bowel wall and mediate the peristaltic reflex (3). Other endings in the mucosa are extrinsic and have their cell bodies in the nodose or dorsal root ganglia, and project into the central nervous system (4). These provide the basis, not only for reflexes, but also for sensations and behavioural responses, including nausea and vomiting. The involvement of vagal afferents in emesis resulting from chemo- and radiotherapy is well documented (5) and with the advent of 5-HT_3 receptor antagonists, which provide symptomatic relief for cancer patients, it is clear that serotonin plays a pivotal role in the vomiting response, at least in the acute phase of treatment (6). However, both peripheral and central actions of 5-HT are implicated in vomiting and for the former the mechanisms that underlie vagal activation are poorly understood. The aim of the present study was to examine the sensitivity of extrinsic afferents to 5-HT, to characterize the receptor mechanisms involved, and to determine the role of endogenous 5-HT, released by cisplatin, on afferent impulse generation.

Recent electrophysiological approaches to tackle this question of the peripheral actions of 5-HT have utilized fibre recording techniques to monitor the afferent impulse traffic emanating from the bowel wall, either by recording from the vagus and splanchnic nerves individually, or by recording at the level of the mesenteric nerve bundles where both populations of fibres converge before entering the bowel wall. In this respect, sub-populations of vagal afferent fibres have been described, which respond functionally to stimulation of the muscle and mucosa (7). These endings can be visualized, using fluorescent labelling techniques, terminating in

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either the gastrointestinal mucosa or the external muscle layers (8). Other terminations within the myenteric plexus are suggested to mediate axon reflexes such that information relayed centrally in these afferents is also available for the genesis of local reflexes. Such reflexes make the study of gastrointestinal afferents more difficult because the stimulus–response properties of afferents will be modulated especially by changes in motor activity. It is important, therefore, to eliminate such secondary effects on afferent impulse traffic when interpreting electrophysiological data.

THE ELECTROPHYSIOLOGICAL APPROACH

We have employed two different experimental models to examine the sensitivity of extrinsic afferents to 5-HT. The first is the urethane-anaesthetized ferret (1.5 g/kg); an animal used extensively for the study of emesis. In these experiments we used fibre dissection of the cervical vagus to obtain single unit recording from afferent fibres supplying the gastroduodenal mucosa (see (9) for details of methodology). However, the yield from these experiments, and the difficulty of maintaining single fibre recordings, forced a second approach, in which we have recorded whole nerve discharge from the mesenteric paravascular nerve bundles supplying segments of rat jejunum (10). The choice of the rat for these studies might at first seem strange, given its inability to vomit. However, it is apparent that the absence of a vomiting reflex in the rat does not result peripherally from a deficit in the vagal afferent pathway but is more likely to be a central phenomenon. In this respect, the rat offers a distinct advantage in that stable recording conditions are not compromised by movement artefacts occurring during an emetic response. Another advantage of mesenteric recordings is that the entire afferent supply to a region of intestine can be sampled simultaneously, and by using waveform discrimination with a CED 1401+ (Cambridge Electronic Design) together with 'Spike' software it was possible to obtain single unit data from several afferents at any one time. Choosing afferents with different response characteristics allows the on-line comparison of mucosal and muscle afferent responses to a variety of stimuli.

THE SPECIFICITY OF 5-HT ACTION ON MUCOSAL AFFERENTS

Vagal mucosal afferents have been characterized as polymodal chemoreceptors on the basis of their mechanosensitivity to mucosal deformation and chemosensitivity to luminal stimulation with acid, hypertonic saline or nutrients (7). These are generally spontaneously silent, but during the course of repeated mucosal stimulation, they often generate a low frequency background discharge, upon which experimentally induced responses are superimposed. In the ferret, the response of single vagal mucosal afferents to systemic 5-HT typically consists of a high

frequency burst of action potentials lasting around 5–10 seconds (Figure 1). The response is dramatic because it often arises out of quiescence, is rapid in onset at a latency consistent with the circulation delay, and generates impulses at frequencies up to about 50 impulses/second, which is as high as one ever observes in these C-fibre afferents. The response to 5-HT has a threshold around 1 μg (intra-arterial) and increases in magnitude and duration with higher doses, although maximum responses are not routinely examined because of the bradycardia that accompanies higher doses of 5-HT. However, the mucosal afferent discharge is not secondary to this Bezold-Jarisch response, as it occurs before the cardiovascular response when delivered intra-arterially, and remains after treatment with atropine and hexamethonium, which would also reduce the motor effects of 5-HT on gastrointestinal muscle. Moreover, the sensitivity to 5-HT is not a nonspecific response of gastrointestinal C-fibre afferents but is present only in mucosal chemoreceptors.

Vagal afferents with endings in the muscle behave as in-series tension receptors, and respond to both passive stretch and active contraction of the gut wall. These afferents respond to 5-HT only indirectly following changes in muscle tone in the vicinity of the receptive field (11). As 5-HT triggers a vagally mediated relaxation of the proximal stomach, afferents with endings in the gastric corpus are off-loaded during this fall in tone and as a result show a reduction in impulse generation following 5-HT. Other mechanoreceptive endings in the antrum and small intestine are stimulated as a consequence of contractions in these regions caused by 5-HT. Thus, the response to 5-HT is specific for vagal mucosal afferents.

Whole nerve mesenteric discharge in the rat follows a similar pattern of response to 5-HT to that described above. However, in these multi-unit recordings, which contain both mechano- and chemo-sensitivity afferents, it is often possible

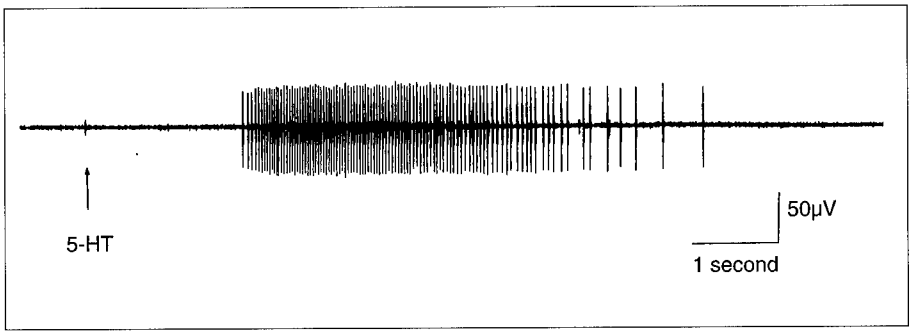

Figure 1. A recording from a single vagal afferent unit in a ferret, illustrating the response of a fibre with a receptive field in the duodenal mucosa to systemic 5-HT (10 μg). Note that 5-HT evoked a powerful burst of action potentials in this previously silent afferent fibre.

to distinguish the short latency direct responses of some afferent fibres from longer duration responses that are secondary to changes in motility. The short latency responses of both vagal and mesenteric afferents to 5-HT are mimicked by the 5-HT_3 receptor agonist, 2-methyl-5-HT, and blocked by the 5-HT_3 receptor antagonist, granisetron (0.5 mg/kg). The delayed response of some mesenteric afferents to 5-HT is mimicked by high dose (200 μg) 5-methoxytryptamine.

Evidence for an action of endogenous 5-HT on these mucosal afferents has been obtained from experiments in which cisplatin (10 mg/kg) was administered. Following cisplatin administration, there is an increase in whole nerve activity which begins after approximately 10 minutes and reaches a plateau by about 30 minutes. Granisetron produces a dramatic reduction in the whole nerve activity, returning nerve discharge to close to control levels. Although the response to cisplatin is therefore mediated by 5-HT acting on the 5-HT_3 receptor, an essential role for 5-HT in the transduction of other stimuli appears unlikely, as these afferents are still responsive to mucosal probing or chemical stimulation, with acid or hypertonic saline, after treatment with granisetron.

IMPLICATIONS FOR SIGNAL TRANSDUCTION

These data on 5-HT actions on extrinsic afferents contrast with Kirshgessner, Tamir and Gershon's study (12) of enteric afferents, which they visualized using c-*fos* expression as an activity marker following luminal mechanical (villus movement) and chemical (cholera toxin) stimulation. The activation of these mucosal enteric afferents was blocked by treatment with a '5-HT_{1P}' receptor antagonist, which the authors interpreted as confirming an obligatory role for 5-HT released from enterochromaffin cells in afferent signal transduction. We have shown that 5-HT also represents a potent stimulus to gastrointestinal afferent fibres projecting to the central nervous system but the receptors involved are of the 5-HT_3 class. Moreover, although these afferents respond to endogenous 5-HT released by treatment with cisplatin, blockade of the 5-HT_3 receptor does not interfere with the ability of these afferents to respond to other stimuli such as mechanical distortion or luminal acid. The time-course of afferent activation by cisplatin differs considerably from the emetic response itself, as typified by that seen in the ferret (5). Thus, afferent firing begins within 10 minutes of administration, whereas vomiting is delayed by about an hour. If these afferents contribute (in an animal capable of vomiting) to the emetic response, then the factors responsible for the delay remain to be resolved. The response of vagal afferents is confined to those terminating within the mucosa as muscle mechanoreceptors respond only indirectly to 5-HT following changes in gastroduodenal motility (10, 11). However, the modulation of impulse generation by 5-HT appears to be just one of the mechanisms that determine the broad chemosensitivity in these fibres, and that others, not involving 5-HT release, must also be involved.

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Chapter 9

The electrophysiology of vagal and recombinant 5-HT_3 receptors

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INTRODUCTION

The 5-HT_3 receptor is a member of the family of ligand-gated ion channels that includes the nicotinic cholinoceptor, $GABA_A$ and glycine receptors. The receptor is widely distributed throughout the peripheral nervous system, occurring on sympathetic, parasympathetic, enteric and sensory elements (1) and is also present at discrete locations within the central nervous system. The activation of 5-HT_3 receptor populations results in rapid depolarizing responses that are contingent upon opening of cation-selective ion channels that, under physiological conditions, primarily conduct Na^+ and K^+ ions. The involvement of the 5-HT_3 receptor in nausea and vomiting evoked by cytotoxic drugs and radiation is firmly established, and current evidence (2) suggests the receptors located upon abdominal vagal afferents play a pivotal role in the initiation of these effects. 5-HT_3 receptors are a ubiquitous component of the plasmalemma of certain primary visceral afferent neurones (C-cells), but it is the receptor populations located at the peripheral and central terminals of vagus nerve that are of greatest relevance in the context of emesis. The electrophysiological characterization of vagal 5-HT_3 receptors can contribute to a better understanding of the events occurring in drug- and radiation-induced emesis but, for practical reasons, investigations have tended to concentrate upon the receptor populations located upon vagal axons and the visceral primary afferent cell bodies contained within the nodose ganglion, rather those present at vagal terminals.

Figure 1 summarizes the distribution of 5-HT_3 receptor populations upon primary visceral afferents and illustrates, in a highly schematic manner, the types of electrophysiological response that can be recorded from such cells. Extracellular recordings from single vagal afferents supplying the upper gastrointestinal tract clearly demonstrate that, in common with C-fibres innervating the cardiopulmonary region and carotid bodies, such fibres are transiently activated by close arterial or topical applications of 5-HT_3 receptor agonists, an effect that can be blocked by selective 5-HT_3 receptor antagonists. Under pathophysiological conditions (administration of cytotoxic drugs or radiotherapy), 5-HT released from gastrointestinal mucosal enterochromaffin cells is envisaged as providing a stimulus that activates, and possibly sensitizes, adjacent C-fibre terminals (2). 5-HT_3 receptors located upon the axons of the isolated and desheathed cervical vagus nerve have long been known to support a depolarizing action of 5-HT, which

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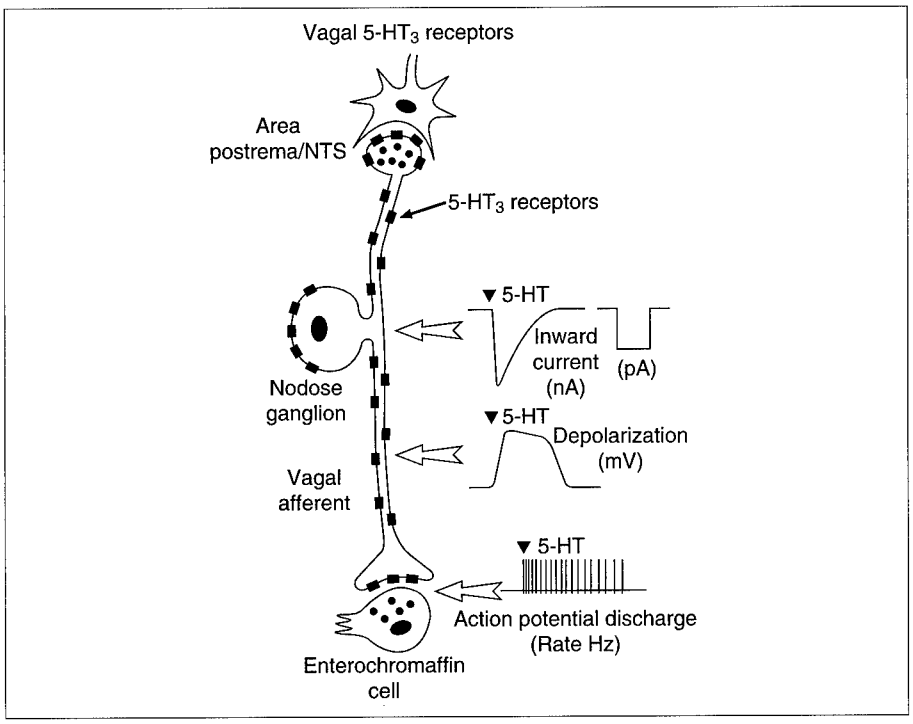

Figure 1. Diagram depicting the distribution of 5-HT_3 receptors on primary visceral afferent neurones. The presence of 5-HT_3 receptors at the peripheral terminals of afferents innervating the upper gastrointestinal tract may be demonstrated by recording spike discharge from single fibres in response to close arterial applications of 5-HT_3 receptor agonists as shown schematically to the right. Axonally located 5-HT_3 receptors mediating membrane depolarization, shown in an idealized manner to the right, are revealed in extracellular recordings performed on the isolated and desheathed cervical vagus nerve. Somatic 5-HT_3 receptors may be demonstrated and studied via a variety of electrophysiological techniques including extra- and intracellular recording and patch-clamp techniques. In the stylized records presented here, macroscopic inward current responses and single-channel currents, which can be obtained in recordings from isolated whole-cells and membrane patches, respectively, are shown. 5-HT_3 receptors on central terminals of the vagus have been demonstrated by a combination of autoradiographic and lesioning techniques, and are considered in Chapters 10 and 11. NTS, nucleus tractus solitarius.

can be recorded using grease- or sucrose-gap recording techniques (1). This assay has been used extensively in the evaluation of 5-HT_3 receptor agonists and antagonists. Detailed investigations of 5-HT_3 receptor function have been conducted upon the vagal afferent cell bodies in isolated nodose ganglia. Here it has been possible to employ intracellular and two-electrode voltage-clamp techniques to

record 5-HT_3 receptor-evoked depolarizations and macroscopic current responses, respectively. Additionally, the use of enzymatically dispersed nodose ganglion neurones maintained *in vitro* has allowed both whole-cell and single-channel currents mediated by 5-HT_3 receptors to be analysed by patch-clamp techniques. The results of such studies form the basis of this chapter.

GENERAL CHARACTERISTICS OF 5-HT_3 RECEPTOR-MEDIATED ELECTRICAL RESPONSES IN NODOSE GANGLION NEURONES

Intracellular recordings performed upon neurones of the rabbit isolated nodose ganglion indicate that the vast majority of C-cell, but not A-cell, somata possess 5-HT_3 receptors (1). In such cells, 5-HT_3 receptor activation elicits a depolarizing response and associated action potential discharge that is essentially phasic in nature. Even in the continued presence of superfused 5-HT, the depolarizing response is limited in duration by the development of receptor desensitization, and is characteristically succeeded by an after hyperpolarization. The latter results from Ca^{2+} influx during the depolarizing response, and the subsequent activation of a Ca^{2+}-dependent potassium conductance (3). Ca^{2+} entry probably largely resides in the activation of voltage-activated calcium channels during the 5-HT-evoked depolarization. Any contribution that might be expected from the 5-HT_3 receptor channel itself, which in nodose ganglion neurones is finitely permeable to Ca^{2+} (4), is probably too small to be of significance when physiological concentrations of ions are present. In agreement with this conclusion, 5-HT_3 receptor activation elicits a simple monophasic inward current response that activates and desensitizes rapidly under voltage-clamp conditions. From a pioneering study of 5-HT_3 receptor-mediated currents recorded in media of altered ionic composition, Higashi and Nishi (3) concluded that the channel integral to the receptor was permeable to Na^+ and K^+ ions but impermeable to Cl^- and Ca^{2+}. More recent studies of 5-HT_3 receptor-activated currents recorded from enzymatically dispersed rabbit nodose ganglion neurones maintained *in vitro*, (5) corroborate the view that the channel is essentially non-selective towards small monovalent metals. Indeed, the reversal potential ($E_{5\text{-}HT}$) of 5-HT_3 receptor-mediated currents has been found, with few exceptions, to be around 0 mV in all neuronal types examined to date, a value consistent with the presence of a non-selective cation-conducting channel (4).

BIOPHYSICAL ANALYSIS OF 5-HT_3 RECEPTORS IN NODOSE GANGLION NEURONES

Ion substitution experiments involving the replacement of extracellular Na^+ with organic monovalent cations of differing molecular sizes (geometric mean diameters), coupled with measurements of $E_{5\text{-}HT}$, have allowed the estimation of the diameter of

the 5-HT_3 receptor channel at its narrowest point (6). Such an analysis applied to the receptor expressed by rabbit nodose ganglion neurones and mouse N18 neuroblastoma cells has revealed values of approximately 8.1 and 7.6 Å, respectively. These diameters are similar to those reported for the nicotinic acetylcholine receptor of the neuromuscular junction, with which the 5-HT_3 receptor shares many common features of structure and function (6).

The conductance of single 5-HT_3 receptor ion channel complexes has been examined in a variety of preparations including outside-out membrane patches excised from rabbit nodose ganglion neurones maintained in culture (5). Figure 2 illustrates the results of a typical experiment using the latter, where the application of 1 μM 5-HT evoked the appearance of multiple single-channel activity that declined in frequency (desensitized) in the continued presence of the agonist. Such unitary currents were susceptible to antagonism by metoclopramide (1 μM). The conductance of the 5-HT_3 receptor channel in rabbit nodose ganglion neurones is approximately 19 pS, a value similar to that (15 pS) reported for the higher of two conductance states found in guinea-pig submucous plexus neurones. However, this value is somewhat higher than that reported for 5-HT_3 receptors expressed in mouse and rat superior cervical ganglion neurones (≈ 10 pS) and guinea-pig coeliac ganglion neurones (≈ 10 pS) and contrasts markedly with the extremely low conductances (≈ 0.3–0.6 pS) found for 5-HT_3 receptors endogenous to the neuronal cell lines N1E–115 and N18 (6). The diversity of channel conductances that have been observed for 5-HT_3 receptors in different locations indicates the existence of either species variants of the receptor or, perhaps, true receptor subtypes within a species (see below).

PHARMACOLOGICAL PROPERTIES OF 5-HT_3 RECEPTORS IN NODOSE GANGLION NEURONES

The pharmacological profile of 5-HT_3 receptors expressed by rabbit nodose ganglion neurones has been studied using both extracellular and voltage-clamp recording techniques. In the voltage-clamp study of Higashi and Nishi (3) conducted upon whole ganglia *in vitro*, 5-HT_3 receptor activation in response to either superfused or iontophoretically applied 5-HT was best described by the cooperative binding of at least two molecules of 5-HT. Subsequently, a cooperative receptor model has been found to describe the kinetics of 5-HT_3 receptors in a wide variety of preparations (4). In common with 5-HT_3 receptors expressed elsewhere, those of rabbit nodose ganglion neurones are activated by the relatively selective agonists 2-methyl-5-HT and 1-phenylbiguanide, albeit with a potency considerably lower than that of 5-HT itself (5). The influence of a range of selective and non-selective antagonists upon inward current responses elicited by locally applied 5-HT has been studied in voltage-clamped nodose ganglion neurones isolated from the rabbit. Figure 2 illustrates the data obtained with ondansetron, applied cumulatively in

increasing concentrations against inward current responses to brief local applications of 5-HT. Inspection of the trace emphasizes the slow recovery from antagonism that is characteristic of high affinity antagonists (e.g. tropisetron, bemesetron) acting upon this preparation. Such a slow recovery implies that equilibrium between agonist and antagonist binding cannot be achieved during the relatively brief time-course of the 5-HT-induced current, which, of course, is limited by the development of receptor desensitization. This 'hemi-equilibrium' condition that prevails can explain, at least in part, why agents such as ondansetron and tropisetron display non-competitive kinetics in many functional assays, yet behave in a manner entirely consistent with competitive antagonism in binding assays conducted at equilibrium (4).

Table 1 provides a summary of the antagonist pharmacology of the 5-HT_3 receptor of the rabbit nodose ganglion determined under the experimental conditions detailed above. A comparison of these data with those previously obtained from murine N1E-115 neuroblastoma cells under identical recording conditions (7) revealed that the apparent potencies of the non-selective agents, cocaine and (+)-tubocurarine, vary markedly between the two preparations. The possibility that a species difference might underlie such diversity was addressed by further experimentation upon the 5-HT_3 receptors expressed by mouse and guinea-pig nodose ganglion cells, and the results of that study are included in Table 1. From such comparisons, it is evident that (+)-tubocurarine effectively discriminates between 5-HT_3 receptors expressed by mouse, rabbit and guinea-pig neurones. Concern might be expressed regarding the mode of action of (+)-tubocurarine, which is capable of exerting uncompetitive antagonism of certain nicotinic receptors through open-channel blockade. However, the available evidence from both functional and radioligand binding assays would tend to favour a competitive interaction between (+)-tubocurarine and the 5-HT_3 receptor (4). In any event, the demonstration of species differences does not rest upon (+)-tubocurarine alone. Cocaine also acts as a discriminatory ligand in the systems tested, and ondansetron clearly shows reduced potency at the guinea-pig 5-HT_3 receptor. Furthermore, species differences can also be demonstrated with putative allosteric modulators. For example, ketamine (4) exerts a biphasic effect upon 5-HT_3 receptors of rabbit nodose ganglion neurones (i.e. potentiation in response to low micromolar concentrations of the drug succeeded by blockade at higher doses), yet produces only a concentration-dependent inhibition at the guinea-pig receptor (Gill, Peters and Lambert, unpublished observations). Similar results have been obtained with trichloroethanol (Gill, Peters and Lambert, unpublished observations).

The studies reported above are consistent with an emerging literature (4) in which species differences in 5-HT_3 receptor pharmacology have been detected in functional and radioligand binding assays. The challenge now is to explain such differences at the molecular level and to address in detail the nature of the receptor in man. The cloning of a 5-HT_3 receptor subunit, initially from the hybridoma cell line NCB-20 (8), and subsequently by homology cloning from other sources, has provided the means to address these issues.

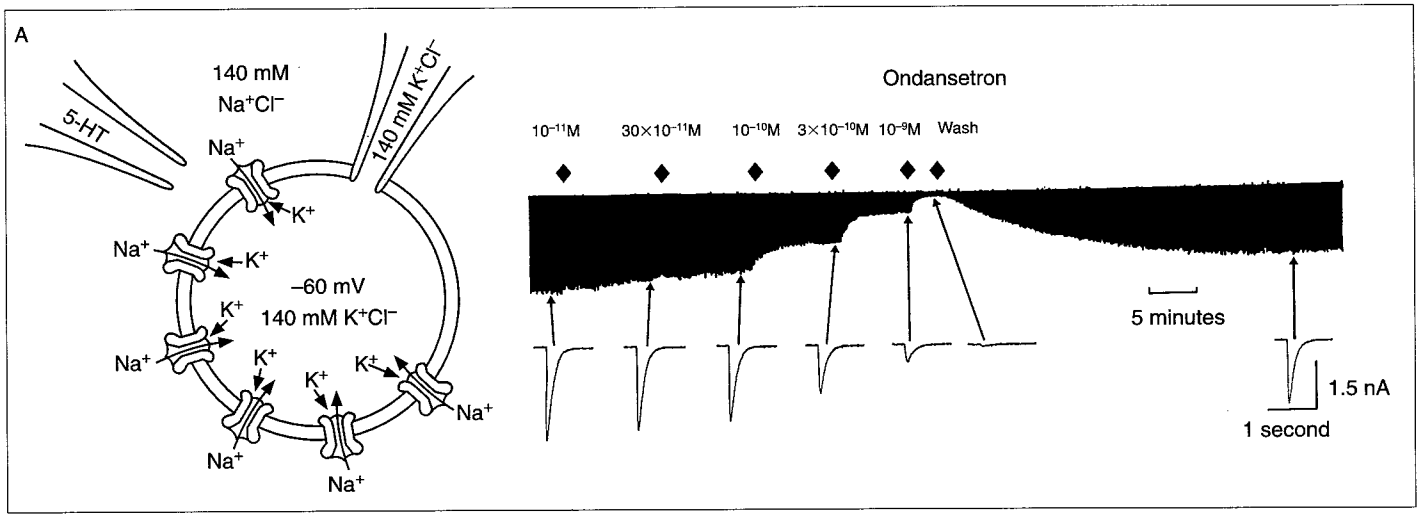

Figure 2. Pharmacological and biophysical properties of 5-HT_3 receptors expressed by rabbit isolated nodose ganglion neurones maintained in cell culture.

A: Left, schematic representation of the whole-cell recording technique used to record macroscopic currrent responses to 5-HT from the ganglion cell soma. Right, time-course of the inhibition by ondansetron of inward current responses to 5-HT. Ondansetron (10^{-11} M–10^{-9} M) produced a concentration-dependent inhibition of currents elicited by brief (20 ms) and repetitive (0.1 Hz) applications of 5-HT (10^{-5} M) by pressure. The upper trace is shown on a compressed time-scale to emphasize the temporal characteristics of both the onset and recovery from antagonism. Individual current responses, which cannot be discerned in the compressed record, are shown on an expanded time-scale in the lower traces. Note the slow recovery from antagonism by ondansetron. Currents were recorded at a holding potential of –60 mV.

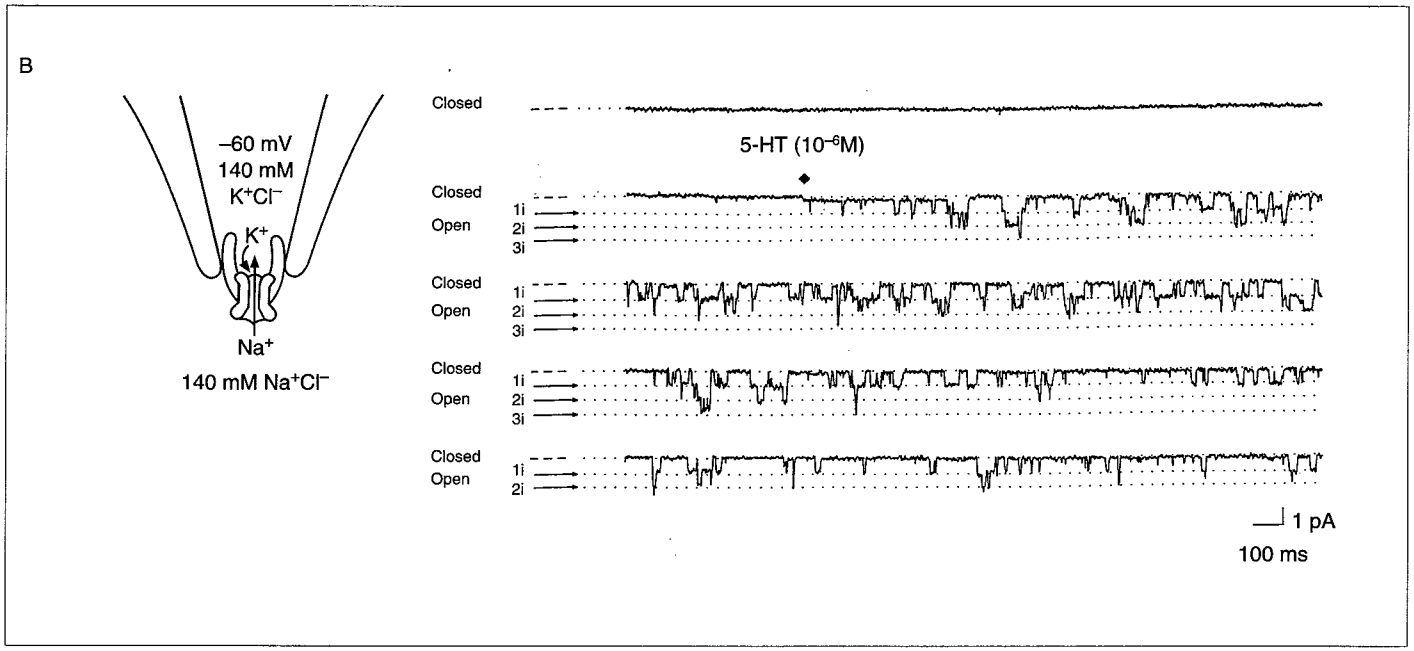

Figure 2. B: Left, schematic representation of the outside-out recording mode of the patch-clamp technique utilized to examine the single-channel properties of 5-HT$_3$ receptors. Right, single-channel activity elicited by the application of 5-HT (1 μM) to an outside-out membrane patch voltage-clamped at a holding potential of –80 mV. Note that this particular patch contained at least three 5-HT$_3$ receptor channel complexes and that single channel activity declines in the continued presence of 5-HT, indicating the onset of receptor desensitization.

Table 1. Summary of the pharmacology of 5-HT_3 receptors endogenous to nodose ganglion neurones and N1E-115 cells and recombinant homo-oligomeric 5-HT_3R-A_s receptors expressed in *Xenopus laevis* oocytes.

	pEC_{50} or pIC_{50} values Rabbit NGN	G-pig NGN	Mouse NGN	N1E-115 cell	Mouse 5-HT_3R-A_s	Human 5-HT_3R-A_s
Agonist						
5-HT	–	–	–	5.4	5.6	5.5
m-CPBG	–	–	–	6.1	6.1	5.4
2-Me-5-HT	–	–	–	4.8	4.8	5.3
PBG	–	–	–	4.7	4.6	4.1
Antagonist						
Ondansetron	10.2	7.7	9.4	9.6	8.9	9.5
Granisetron	–	–	–	9.9	9.8	9.5
BRL 46470	–	–	–	–	9.8	9.1
Metoclopramide	7.9	5.9	–	7.6	7.1	5.7
Cocaine	7.1	5.7	5.3	5.1	5.6	6.1
(+)-Tubocurarine	6.8	5.0	8.8	9.1	8.8	5.6

Data are expressed as the negative logarithm of the concentration (in molar units) of agonist required to evoke a half-maximal current response (i.e. pEC_{50}) or the negative logarithm of the concentration of antagonist producing half-maximal inhibition (i.e. pIC_{50}). The data have been assembled from references 4, 5, 6, 9 and 10 and additionally include the unpublished observations of the authors made on the human 5-HT_3R-A_s subunit expressed in *Xenopus* oocytes. All data are from voltage-clamp studies performed on the preparation indicated. Abbreviations: 2-Me-5-HT, 2-methyl-5-HT; *m*CPBG, *meta*-chlorophenylbiguanide; PBG, 1 phenylbiguanide; G-pig, guinea-pig; NGN, nodose ganglion neurone.

THE PROPERTIES OF CLONED 5-HT_3 RECEPTOR SUBUNITS

The 5-HT_3 receptor subunit (5-HT_3R-A) cloned from murine NCB-20 cells demonstrates sequence similarity to other ligand-gated ion channels, particularly certain nicotinic receptor subunits, and is suggested to have the topological organization proposed for other members of this family; namely four transmembrane spanning regions connected by extra- and intracellular loops and a large extracellular domain containing the ligand binding site (8). Evidence for the latter assertion has been obtained from the construction of chimeric constructs of nicotinic and 5-HT_3R-A subunits, the pharmacological properties of which are dictated by the receptor species contributing the extracellular N-terminal domain (reviewed in 6). 5-HT_3R-A subunits, or more precisely splice variants termed 5-HT_3R-A_S, have been cloned from murine N1E-115 neuroblastoma cells, NG108-15 hybridoma cells, rat superior cervical ganglion neurones (6), and most recently a human amygdala cDNA library (unpublished observations). These cloned subunits demonstrate a very high degree of amino acid sequence identity and as such represent species homologues of a common 5-HT_3 receptor subunit. 5-HT_3R-A_S subunits assemble efficiently in heterologous expression systems, such as *Xenopus laevis* oocytes and HEK 293 cells, to yield functional homo-oligomeric complexes that represent the properties of native 5-HT_3 receptors to a remarkable extent. Figure 3 presents examples of the effect of (+)-tubocurarine upon 5-HT-evoked inward current responses, recorded under voltage-clamp from *Xenopus* oocytes expressing either the mouse or the human recombinant 5-HT_3R-A receptor. A summary of a more complete pharmacological analysis of the two species homologues can be found in Table 1. Several features of these data warrant comment. Firstly, within the boundaries of the compounds thus far examined, the pharmacological properties of the mouse 5-HT_3R-A_S faithfully mirror those of 5-HT_3 receptors endogenous to both murine N1E-115 cells and mouse nodose ganglion neurones. Secondly, although differences in the potencies of agonist compounds acting at the human and mouse homologues of the 5-HT_3R-A_S appear modest, they are nonetheless sufficient to result in an altered rank order of effectiveness. Finally, amongst the antagonists tested, (+)-tubocurarine clearly discriminates between the human and mouse 5-HT_3R-A_S subunits, being almost 1800-fold more effective as a blocker of the latter.

The marked discrepancy in the potency of (+)-tubocurarine at human and mouse 5-HT_3 receptors might be viewed as a pharmacological curiosity with no tangible application. However, the surprising discriminatory properties of this ligand may prove to be of assistance in identifying specific amino acid residues that contribute to, or impact upon, the ligand binding site(s) of the 5-HT_3 receptor. Relatively few differences in primary amino acid sequence exist between human and mouse 5-HT_3R-A_S subunits within the extracellular N-terminal domain, yet some of these must account for the selectivity of (+)-tubocurarine for mouse 5-HT_3 receptors and other, more subtle, differences in pharmacology between species homologues. The elucidation of the residues involved is currently being investigated by site-directed mutagenesis.

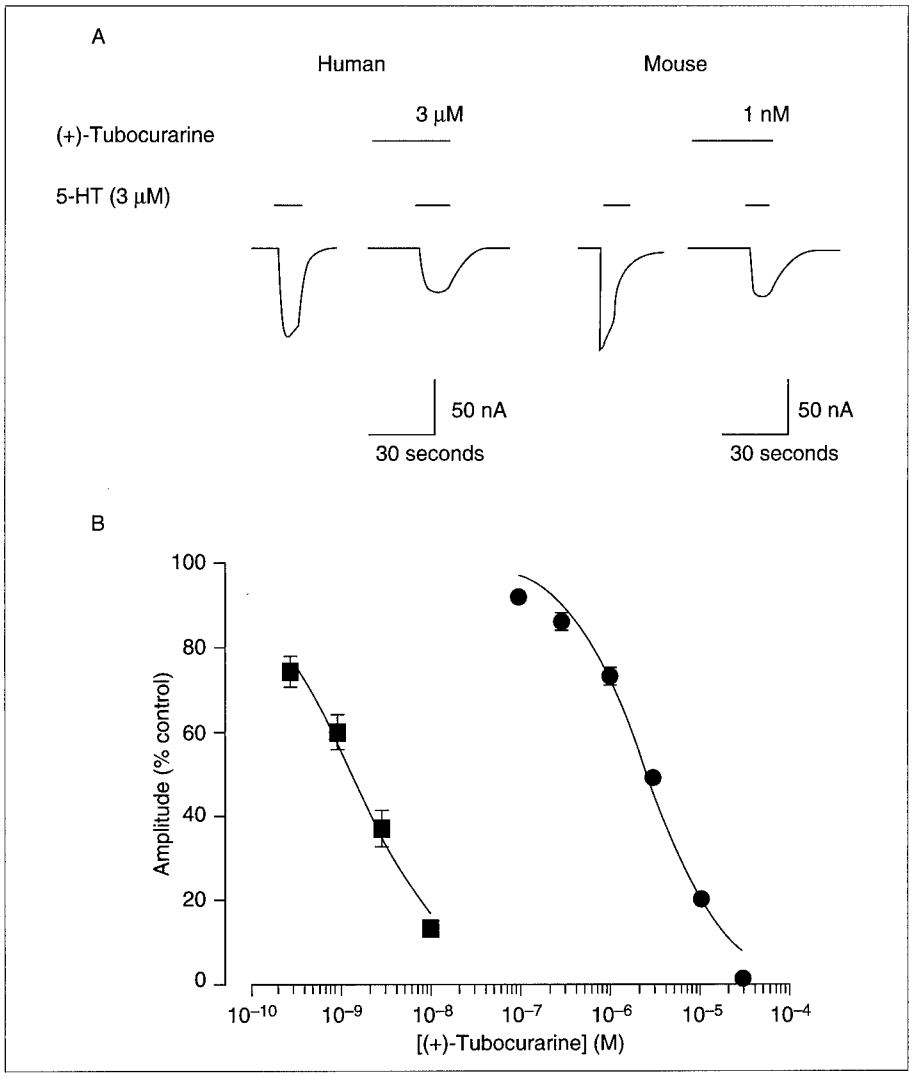

Figure 3. A comparison of the blocking action of (+)-tubocurarine on the human and mouse 5-HT_3R-A_S subunits expressed in *Xenopus laevis* oocytes.

A: Inward currents evoked by bath-applied 5-HT (~EC_{50}) are reduced by ~50% by 3 μM and 1 nM (+)-tubocurarine in the human and mouse 5-HT_3R-A_S subunits, respectively.

B: Graphical depiction of the concentration-dependent inhibition of 5-HT-evoked currents by (+)-tubocurarine in oocytes expressing the human (IC_{50} = 2.6 ± 0.2 μM) and the mouse (IC_{50} = 1.4 ± 0.2 nM) 5-HT_3R-A_S subunits. The relationship between the concentration of the antagonist (log scale x-axis) is plotted against the inhibition of the 5-HT-evoked current (expressed as a percentage of the control value to 5-HT, y-axis). All currents were recorded from oocytes voltage clamped at –60 mV.

ACKNOWLEDGEMENTS

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Chapter 10

5-HT_3 receptors in the dorsal vagal complex

P. A. Brooks and A. P. Albert

INTRODUCTION

Antagonists of 5-hydroxytryptamine$_3$ (5-HT_3) receptors are of proven benefit in controlling emetic responses to cancer chemo- and radiation therapy (1). This effect may result from peripheral mechanisms of action, but a central component can also be postulated. 5-HT_3 receptors are located at several sites along the emetic pathway(s), for example, at the peripheral endings of the afferent fibres, along the length of the afferent fibres, at the site of termination of these afferents in the nucleus tractus solitarius (NTS), and in the dorsal vagal motor nucleus (DMV) (see Chapter 11). We have used an *in vitro* rat brainstem slice preparation to investigate the actions of serotonin (5-HT) in both the NTS and DMV. The functions of the NTS and DMV neurones we have recorded from *in vitro* have not been identified and the rat is not a species with an emetic reflex. However, 5-HT_3 receptors are also involved in the brainstem control of the cardiovascular system (2), and the consistency of our results between neurones implies that several autonomic control systems are likely to be affected.

THE ACTIONS OF 5-HT IN THE DORSAL VAGAL COMPLEX OF THE RAT

In the experiments described, rats (15–25 days of age) were given anaesthetic overdoses and, following dissection of the brainstem, transverse slices (150–250 μm) containing the NTS and DMV were prepared using a vibroslice (Campden Instruments, UK). Whole-cell patch recordings (in more recent studies from visualized DMV and NTS neurones) were made from NTS and DMV neurones, and drugs were applied in the bathing medium at flow rates of 3–4 ml/minute, which effectively caused exchange of perfusate in 1.5 minutes. In addition, synaptic responses were evoked by electrical stimulation of the tractus solitarius (TS) area where primary afferents terminate.

5-HT actions in the rat NTS *in vitro*

In a previous study (3), we reported the direct and indirect effects of 5-HT and 5-HT_3 receptor activation in the NTS. 5-HT responses were predominantly excitatory

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in the NTS, with depolarizations and initiation of action potential firing. Further analysis of these responses in the NTS using selective agonists and antagonists revealed that postsynaptically-mediated depolarizing responses could be obtained with 5-HT_2 and 5-HT_3 ligands (α-methyl-5-HT and 2-methyl-5-HT respectively). The postsynaptic 2-methyl-5-HT (100 μM) responses were effectively antagonized by ICS205-930 (10 nM) and MDL72222 (10 μM). In addition to the postsynaptically mediated depolarizing responses, a large increase in the amplitude and frequency of spontaneous excitatory and inhibitory postsynaptic potentials (spEPSPs/spIPSPs) was frequently observed. However, evoked excitatory and inhibitory PSPs (evEPSPs/evIPSPs) were reduced by 2-methyl-5-HT. Both the increases in spPSPs and the decreases in evPSPs could be obtained at much lower 2-methyl-5-HT concentrations (500 nM–1 μM) than the postsynaptic responses. The evEPSPs and spEPSPs could be produced by transmitter release from the TS afferent terminals or from local excitatory interneurones, whereas the GABAergic spIPSPs and evIPSPs emanate entirely from local inhibitory interneurones, as primary afferent input is purely excitatory. Evoked excitatory PSPs produced by stimulation of the TS, which contains afferent fibres, and spEPSPs, were both mediated by excitatory amino acid receptors (EAAR), as they were abolished by 6-cyano-7 nitroquinoxaline-2, 3-dione (CNQX), and both spIPSPs and evIPSPs were mediated by $GABA_A$ receptors, which were blocked by bicuculline. In the amygdala (4), Sugita *et al.* (1992) have identified a 5-HT_3 receptor-mediated fast EPSP, and in the nucleus prepositus hypoglossi, a slow EPSP, mediated by 5-HT_2 receptors, can be evoked (5). In the NTS, despite evidence for both afferent (6) and central 5-HT projections (7), we have not detected EPSPs that are not produced by EAAR activation. In this context, it is interesting that the non-NMDA EAAR antagonists, CNQX and 6-nitro-7-sulphamobenzo(f)quinoxaline-2, 3-dione (NBQX), have been reported to be anti-emetic in ferrets given cisplatin (8). As the transmitter of vagal afferents at their first synapse in the NTS *in vivo* and *in vitro* (3) acts at EAAR, this implies that activation of these afferents is a necessary prerequisite for cisplatin-induced emesis.

In our studies of the effects of 5-HT in the NTS, several consequences of 5-HT_3 receptor activation were found:

1) a direct, postsynaptic excitation of NTS neurones;
2) a large increase in the frequency and amplitude of both excitatory and inhibitory PSPs;
3) a profound decrease in the amplitude of both excitatory and inhibitory PSPs.

5-HT_2 receptor activation also caused postsynaptic excitation and increased the frequency and amplitude of EPSPs. We did not find evidence for 5-HT_{1A} receptor-mediated effects in the NTS.

5-HT effects in the rat DMV *in vitro*

The major effect of 5-HT in our studies of the DMV *in vitro* is a postsynaptically mediated depolarization/excitation caused by activation of 5-HT_2 receptors. There is,

in addition, a component of this 5-HT-induced excitation that is mediated by 5-HT_3 receptors. Thus 2-methyl-5-HT (100–300 μM) depolarized DMVs (Figure 1) and MDL72222 (10 μM) partially blocked responses to 5-HT. Both 5-HT_2 receptor and 5-HT_3 receptor activation (Figure 1) cause increases in spEPSPs and spIPSPs, but their effects on evPSPs have not yet been studied. In some neurones, a biphasic inhibitory/excitatory response was seen.

DISCUSSION

The effects of 5-HT on central neurones that may be involved in the emetic response are complex. Even at the level of one receptor subtype (e.g. the 5-HT_3 receptor), there are multiple effects involving both pre- and postsynaptic sites. Postsynaptically, 5-HT_2 receptor and 5-HT_3 receptor activation results in depolarizing, excitatory responses in NTS and DMV neurones. Presynaptically, 5-HT_2 receptor and 5-HT_3 receptor activation induces increased spEPSP and spIPSP frequency and amplitude in NTS and DMV cells but a decrease in the size of evPSPs in NTS neurones. The presynaptic effects of 5-HT_3 receptor activation in the brainstem are thus rather different from those in the hippocampal CA1 (9) and dentate gyrus (10) regions, where only inhibitory interneurones are excited, leading to an increase in spontaneous inhibitory synaptic events in principal neurones (although the effects on evoked synaptic events have not been ascertained in these studies). Results of *in situ* hybridization of 5-HT_3 receptor-mRNA studies (11) are consistent with the localization of 5-HT_3-receptors on inhibitory interneurones in the hippocampus.

In the brainstem, the presynaptic effects of 5-HT_3 receptor activation are consistent with a presynaptic depolarizing action that could both increase

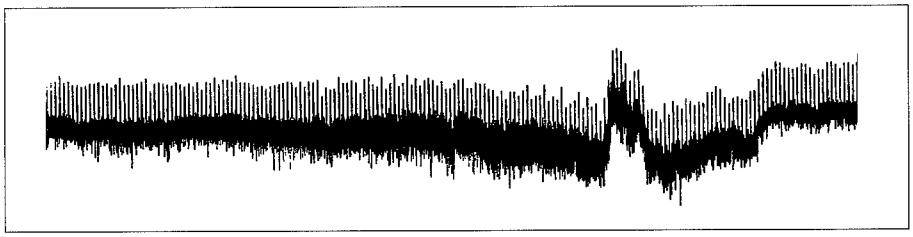

Figure 1. A typical response to the 5-HT_3 receptor agonist, 2-Methyl-5-HT, applied for the duration of the bar, in a visualized dorsal vagal neurone (parasympathetic preganglionic neurone), recorded by the whole-cell patch technique in current clamp mode. Large, regular, downward deflections result from current injections. 2-Methyl-5-HT caused a membrane depolarization and a large increase in spontaneous synaptic activity (increase in baseline thickness), which consisted of both excitatory (upward) and inhibitory (downward) postsynaptic potentials.

spontaneous synaptic activity while reducing evoked release. Interestingly, a similar effect of 5-HT on GABA release has also been reported to occur in the ileum (12). However, although in that tissue the receptor type responsible for the increased spontaneous [^{3}H]-GABA release was 5-HT_3, that responsible for reduction of evoked release was of the 5-HT_{1A} type. Our work on the ability of presynaptic 5-HT_3 receptor activation to affect inhibitory as well as excitatory synapses provides physiological evidence for the location of 5-HT_3 receptors on NTS neurones, in addition to the autoradiographic evidence for their location on vagal afferents. However, in the mouse, *in situ* hybridization studies (11) in the dorsal vagal complex have not shown neurones expressing 5-HT_3 receptor mRNA in this area. The ability of 5-HT_3 receptor activation to influence local inhibitory neurones in NTS and DMV is thus inconsistent with the neurophysiological findings.

In our studies, we have not discerned responses mediated by 5-HT_{1A} receptors, although presynaptic effects have not yet been evaluated.

The ability of 5-HT_3 receptor antagonists to inhibit emesis in the central nervous system may result from the presynaptic reduction of excitatory inputs from vagal afferents, thus blocking the emetic reflex. This hypothesis is in agreement with the ability of excitatory amino acid antagonists to inhibit emesis (8).

ACKNOWLEDGEMENTS

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Chapter 11

Where do 5-HT_3 receptor antagonists act as anti-emetics?

D. J. M. Reynolds

INTRODUCTION

In the last decade, the emergence of 5-HT_3 receptor antagonist drugs has made a substantial contribution towards the alleviation of nausea and vomiting caused by anti-cancer therapy, and has provided a useful additional approach for the management of postoperative emesis. The question of where 5-HT_3 receptor antagonists act as anti-emetics is an important one in any attempt to understand the pathophysiology of emesis, and hence in the development of new anti-emetic drugs. The 5-HT_3 receptor antagonists in current clinical usage are highly selective, and although there are some differences between them in their basic pharmacology, there are no fundamental clinical differences (see Chapter 17), and there is no reason to suppose that they exert their anti-emetic effects through substantially different sites or mechanisms.

Our understanding of the pathways involved in emesis owes much to the work of Borison and Wang (1), but through no fault of theirs, the framework that they proposed to explain emetic circuitry has subsequently been misinterpreted (see Chapter 1, Figure 2). The identification of the so-called 'chemoreceptor trigger zone' for vomiting, in the area postrema gave rise to the concept of central *versus* peripheral emetic stimuli, and this polarization of thought has led to much confusion. In addition, the relative contributions of central or peripheral sites have been determined largely by lesioning studies, and the implicit (and erroneous) assumption that the coordination of the emetic reflex is a static process, unaffected by neuronal adaptation (2). When evaluating the information available, it is important to appreciate that the sites of action of anti-emetic drugs are not necessarily the same as the sites at which the emetic stimulus acts. Furthermore, the observation that acute, but not delayed, emesis following cisplatin therapy is responsive to 5-HT_3 receptor antagonism suggests that either different emetic mechanisms are important at different times after an emetic insult, or that adaptive changes in the emetic pathways may occur relatively rapidly.

I shall make the (not unreasonable) assumption that 5-HT_3 receptor antagonist drugs are exerting an effect only through actions at 5-HT_3 receptors, and shall look at the currently available evidence for the distribution of 5-HT_3 receptors where clinically relevant actions of 5-HT_3 receptor antagonists might be anticipated (3). I shall also limit the discussion to cytotoxic drug-induced emesis, as most experimental and clinical data have been gained with these agents.

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5-HT_3 RECEPTORS IN THE GUT

In the gut, 5-HT_3 receptors are found in association with the intrinsic enteric nervous system, enterochromaffin cells, and the extrinsic fibres of the vagus and splanchnic nerves. Although there is a large literature relating the functional aspects of 5-HT_3 receptors in the gut (4) there are less data available concerning their precise location.

There are numerous human and animal studies that suggest that emesis caused by cytotoxic drugs involves the release of 5-HT in the gut, and that a major source of this 5-HT is enterochromaffin cells (see Chapters 6, 7 and 14). The precise mechanism whereby cytotoxic drugs cause 5-HT release from enterochromaffin cells is unknown. As they are innervated by both vagal and enteric neurones, it is conceivable that emetic cytotoxic drugs act to influence the neuronal input to enterochromaffin cells. Hence, 5-HT_3 receptors within the enteric nervous system are a possible target for the 5-HT_3 receptor antagonists. 5-HT_3 receptors are also located on enterochromaffin cells themselves, and appear to play a role in the regulation of 5-HT release (see Chapter 7). However, patients who receive cisplatin-based chemotherapy and anti-emetic cover with a 5-HT_3 receptor antagonist, and who do not develop emesis, still show a rise in urinary 5-HIAA comparable to that seen in patients who do vomit (5, 6). This is in contrast to patients undergoing radiotherapy, in whom rises in urinary 5-HIAA following treatment are reduced by ondansetron (see Chapter 13). In addition, not all cytotoxic drugs appear to cause 5-HT release from enterochromaffin cells. For example, cancer patients treated with cyclophosphamide or doxorubicin develop emesis that is inhibited by 5-HT_3 receptor antagonists, but which is not associated with the elevation of plasma chromogranin or urinary 5-HIAA seen with other cytotoxic drugs (see Chapter 14). It seems, therefore, that direct or indirect inhibition of 5-HT release from enterochromaffin cells may not be universally essential to the anti-emetic actions of 5-HT_3 receptor antagonists.

Following administration of cisplatin at least, it is clear that 5-HT is released from enterochromaffin cells into the proximity of vagal afferent fibres, and this is very elegantly demonstrated by electron microscopy (see Chapter 5). Vagal afferent depolarization by 5-HT occurs through 5-HT_3 receptors, and despite a lack of conclusive evidence, it has become widely accepted that it is at these vagal terminals in the gut that anti-emetic 5-HT_3 receptor antagonist drugs act. Vagal afferent firing begins within 10–20 minutes of administration of cisplatin (see Chapter 8), whereas vomiting is delayed by about an hour. However, in other studies, vagal afferent activity does correlate well with the time of onset of emesis (see Chapter 5).

5-HT_3 RECEPTORS AND THE VAGUS NERVE

5-HT_3 receptors can be readily identified on desheathed vagus nerve and nodose ganglion preparations, using extracellular recording techniques (7, 8). In addition,

radioligand binding studies have demonstrated 5-HT_3 receptor binding sites in membranes of cat, rat and rabbit vagus nerve (9, 10). Hoyer *et al.* (9), using autoradiography, demonstrated high densities of 5-HT_3 receptor binding in the nodose ganglion and along the length of the cervical vagus nerve of the cat. The 5-HT_3 receptor binding sites in the nodose ganglion were observed over the somata of the pseudounipolar vagal afferents. The role of apparently functional receptors along the length of a nerve trunk, and not at synaptic sites, is difficult to understand, but they are unlikely to be a significant site of action of 5-HT_3 receptor antagonist drugs *in vivo*.

5-HT_3 RECEPTORS IN THE BRAINSTEM

At the end of 1987, with the development of tritiated 5-HT_3 receptor ligands, Kilpatrick and colleagues first reported specific 5-HT_3 receptor binding sites in membranes derived from different regions of rat brain (11). Soon afterwards, two groups reported the regional distribution of 5-HT_3 receptors using quantitative autoradiography (12, 13). Both groups identified high densities of specific binding in the brainstem. Waeber and colleagues described dense binding of [^{3}H] tropisetron in the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus nerve, and in the spinal trigeminal nucleus in the mouse, and concluded that "this distribution, taken together with the electrophysiological and pharmacological data, suggests a presynaptic localization on sensory nerve terminals" (13). 5-HT_3 receptor binding in human brain was reported around the same time (14). The autoradiographic appearances were remarkable in that 5-HT_3 receptor binding was so highly localized to the dorsal vagal complex, and despite any post-mortem change that might have affected the area postrema, it was apparent that the most dense region of binding was within the dorso-medial NTS in the area that corresponds to the subnucleus gelatinosus, and not the area postrema (14). The subnucleus gelatinosus, is the target site of the heaviest projection of vagal afferent fibres and terminals. In the cat at least, the majority, if not all of these fibres are gastric in origin (15, 16). The other regions of the NTS, as well as the area postrema and the dorsal motor nucleus of the vagus nerve, all receive a lighter vagal projection than the subnucleus gelatinosus (17).

The cat dorsal vagal complex provides the clearest evidence for the distribution of 5-HT_3 receptor binding sites within the subnuclei of the NTS, the dorsal motor nucleus of the vagus nerve, the area postrema and the spinal trigeminal nucleus (Figure 1), (18). In this species there is a very high density of receptor sites in the subnucleus gelatinosus with lower values in the commissural nucleus of the NTS and in the area postrema (Figure 1).

The observation that the highest density of 5-HT_3 receptor binding sites is in the subnucleus gelatinosus of the NTS is supportive of the idea put forward by Waeber and colleagues (13) that 5-HT_3 receptors might be associated with vagal afferent

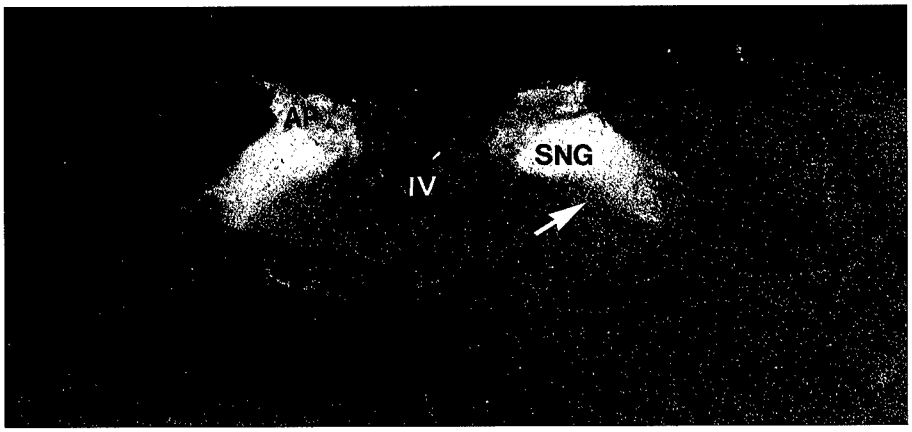

Figure 1. Negative image of an autoradiogram generated over a section of the caudal brainstem of a cat. The section was incubated with 1 nM [^{3}H] GR 65630. Note the very dense binding in the subnucleus gelatinosus (SNG) of the NTS and the much lighter binding in the other subnuclei and in the area postrema (AP). The arrow indicates an area of low level binding in the dorsal motor nucleus of the vagus nerve. IV, fourth ventricle.

terminals. In order to investigate this possibility, we analysed 5-HT$_3$ receptor binding in the ferret dorsal vagal complex in animals that had previously undergone sham operations, unilateral lesions of the cervical vagus nerve, total subdiaphragmatic vagotomy or splanchnic nerve section (19).

Following unilateral cervical vagotomy, binding of [^{3}H] granisetron in the ipsilateral NTS is reduced by two thirds compared with controls, and the effect of the lesion is most marked in the more rostral parts of the NTS. In the caudal parts of the nucleus, the diminution of binding ipsilateral to the lesion is less apparent. Reduction in binding is also seen in the ipsilateral area postrema. Very similar observations have also been made in the rat (20, 21). If a unilateral cervical nerve lesion is combined with dorsal hemisection of the NTS, no binding is seen in the caudal or rostral NTS ipsilateral to the nerve lesion (20).

These observations are consistent with a presynaptic localization of 5-HT$_3$ receptors on vagal afferent terminals in the NTS and area postrema. The lesser effect of a unilateral vagal lesion on 5-HT$_3$ receptor binding in the caudal (compared with the rostral) dorsal vagal complex in the ferret and rat is consistent with the extent of the cross-over of fibres from the contralateral nerve in the more caudal regions. This is strongly supported by the combined effects of dorsal hemisection and vagotomy performed by Pratt and Bowery (20).

Animals subjected to bilateral subdiaphragmatic vagotomy and allowed to recover for 18 days (sufficient to allow transganglionic degeneration to occur) (22) have no 5-HT$_3$ receptor binding sites remaining in the dorsal vagal complex (19). The fibres removed by this lesion are afferents serving abdominal viscera, and

cardiopulmonary afferents should not be affected. This suggests that most, if not all, 5-HT_3 receptors are present on terminals of the vagus nerve that are gastrointestinal in origin. In contrast, splanchnic nerve lesions have no effect on brainstem 5-HT_3 receptor distribution (19).

A vagal terminal distribution of 5-HT_3 receptors is supported by the observation that 5-HT_3 receptor mRNA cannot be detected in the dorsal vagal complex using *in situ* hybridization (23). However, whole-cell patch-clamp recordings from NTS brain slices, although confirming the autoradiographic findings that the majority of 5-HT_3 receptors are presynaptic, suggest that there may be a small number that are postsynaptic ((24); see also Chapter 10).

EVIDENCE FOR A 'CENTRAL' SITE OF ACTION OF CYTOTOXIC DRUGS

Species variations, different experimental paradigms, the use of different drugs, the (im)precision of lesioning studies, and the probable confounding difficulties of plasticity or adaptation of the emetic reflex after lesioning, have produced confusing data on the relative importance of central and peripheral pathways in cytotoxic drug-induced emesis.

As far as cytotoxic drug-induced emesis is concerned, the role of the area postrema is far from clear. In the cat and dog, ablation of the area postrema renders animals refractory to cisplatin-induced emesis but not to cyclophosphamide (25–27). In the ferret, there is recent evidence that ablation of the area postrema inhibits emesis caused by a variety of drugs including cisplatin (Watson, Kovacs and Andrews, personal communication; see Chapter 2).

Subdiaphragmatic vagotomy in the ferret also significantly reduces the emetic response to a variety of cytotoxic drugs, including cisplatin (28, 29). Vagal denervation abolishes the emetic response to cyclophosphamide or cisplatin given intravenously (i.v.) and significantly reduces retching and vomiting when these drugs are administered intraperitoneally (i.p.) (28, 29). Splanchnic nerve denervation alone has no effect on cytotoxic drug-induced emesis but augments the inhibitory effect of vagotomy. It would appear therefore that the integrity of either the area postrema or the visceral afferents is necessary for cytotoxic drug-induced emesis.

In 1988, Smith and colleagues (30) reported a series of experiments in cats, which demonstrated that cisplatin was emetic when administered by intracerebroventricular (i.c.v.) injection, and furthermore, this emetic effect could be inhibited by the 5-HT_3 receptor antagonist, zacopride, given either intravenously or intracerebroventricularly (see below). This study has been frequently quoted to support the contention that cisplatin acts as a 'central' emetic. Conscious cats were administered large doses of cisplatin through chronically in-dwelling intracerebroventricular cannulae (0.3 mg). At this dose, emesis

occurred at a mean time of 4 minutes after injection, compared with 100 minutes when given intraperitoneally. The authors argued that the prolonged latency to emesis when the drug was given by the intraperitoneal or intravenous routes was a reflection of the poor penetration of the drug across the blood-brain barrier, but when given intracerebroventricularly the drug gains almost immediate access to emetic nuclei. Despite the fact that the area postrema has a deficient blood-brain barrier and should therefore not be subject to any such a delayed action of cisplatin, Smith *et al.* (30) offered the hypothesis that cisplatin exerts its central emetic action via the area postrema. The main difficulty with the conclusions of this experiment is that the local concentration of cisplatin achieved in the fourth ventricle with an intracerebroventricular injection of 0.3 mg is likely to have been very high, certainly considerably in excess of that which would result from the peripheral administration of the intraperitoneal dose of 7.5 mg/kg that they used. In view of the very short latency to emesis, it is possible that high concentrations of cisplatin within the area postrema elicit emesis through different mechanisms from those that prevail during clinical use of cytotoxic drugs.

Another approach that has been used to identify the brainstem nuclei involved in emesis is Fos immunocytochemistry (31). Cisplatin administration to ferrets results in Fos protein production in the dorso-medial NTS and the area postrema. In animals that have undergone a previous unilateral cervical vagotomy, the Fos response in the NTS is abolished ipsilateral to the lesion, suggesting that Fos production in this region occurs as a result of vagal afferent input (31). Pretreatment with a 5-HT_3 receptor antagonist (either ondansetron or granisetron) similarly abolishes Fos protein immunoreactivity in the NTS (Figure 2). This is consistent with the evidence from autoradiography that 5-HT_3 receptors are located on vagal afferent terminals. In contrast, induction of the c-*fos* gene in the area postrema following cisplatin treatment is not affected by either vagotomy or 5-HT_3 receptor antagonists, suggesting that cisplatin is having a direct effect on the area postrema. The lack of effect of vagotomy on area postrema Fos immunoreactivity suggests that this Fos protein in the area postrema is not related to the activity of the population of vagal afferents that terminate in the area postrema. Cisplatin can cause c-*fos* gene induction in ependymal cells and other non-neuronal tissue (31, 32) and in our first report of the effect of cisplatin on the area postrema, we could not rule out that these effects were non-specific and unrelated to emesis. In order to address that, we have subsequently used immunocytochemistry with electron microscopy, and it is clear that Fos protein immunoreactivity is present within neurones in the area postrema, and not in other cellular elements (Figures 3 and 4). These results suggest that cisplatin has a direct effect on neurones within the area postrema that is not affected by vagal afferent input or 5-HT_3 receptor antagonism. This supports the lesioning experiments that show the importance of both vagal afferents and the area postrema in cisplatin-induced emesis in the ferret, and is consistent with the data from intracerebroventricular injections of cisplatin.

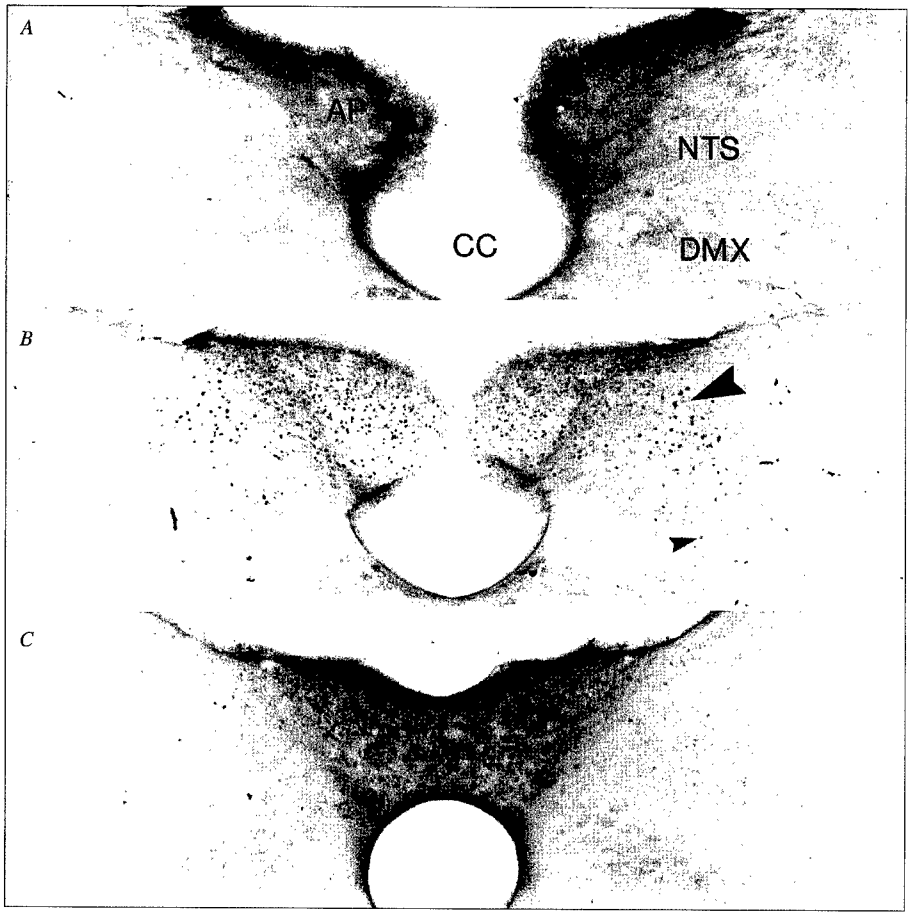

Figure 2. Immunostained sections taken at the level of the mid area postrema.
A: from a ferret that has received saline subcutaneously (s.c.) followed by saline intraperitoneally (i.p.) 30 minutes later. There is a complete absence of Fos immunostaining in this section. The area postrema stains more darkly than surrounding tissue because of non-specific deposition of reaction products within the perivascular spaces (see Figure 3A). CC, central canal; AP, area postrema; NTS, nucleus tractus solitarius; DMX, dorsal motor nucleus of the vagus nerve.
B: from a ferret that received saline s.c. followed by cisplatin 10 mg/kg i.p. Vomiting occurred 75 minutes later and the animal was perfused 2 hours after the onset of emesis. Note the dense Fos immunoreactivity in the area postrema and in the subnucleus gelatinosus (large arrowhead) and medial NTS. A few cells within the DMX also show immunoreactivity (small arrowhead).
C: from a ferret that received granisetron 1 mg/kg s.c. followed 30 minutes later by cisplatin 10 mg/kg i.p. It did not vomit, and was perfused 195 minutes after injection of cisplatin. Note the dense Fos immunostaining in the area postrema but an almost complete lack of staining in the NTS.

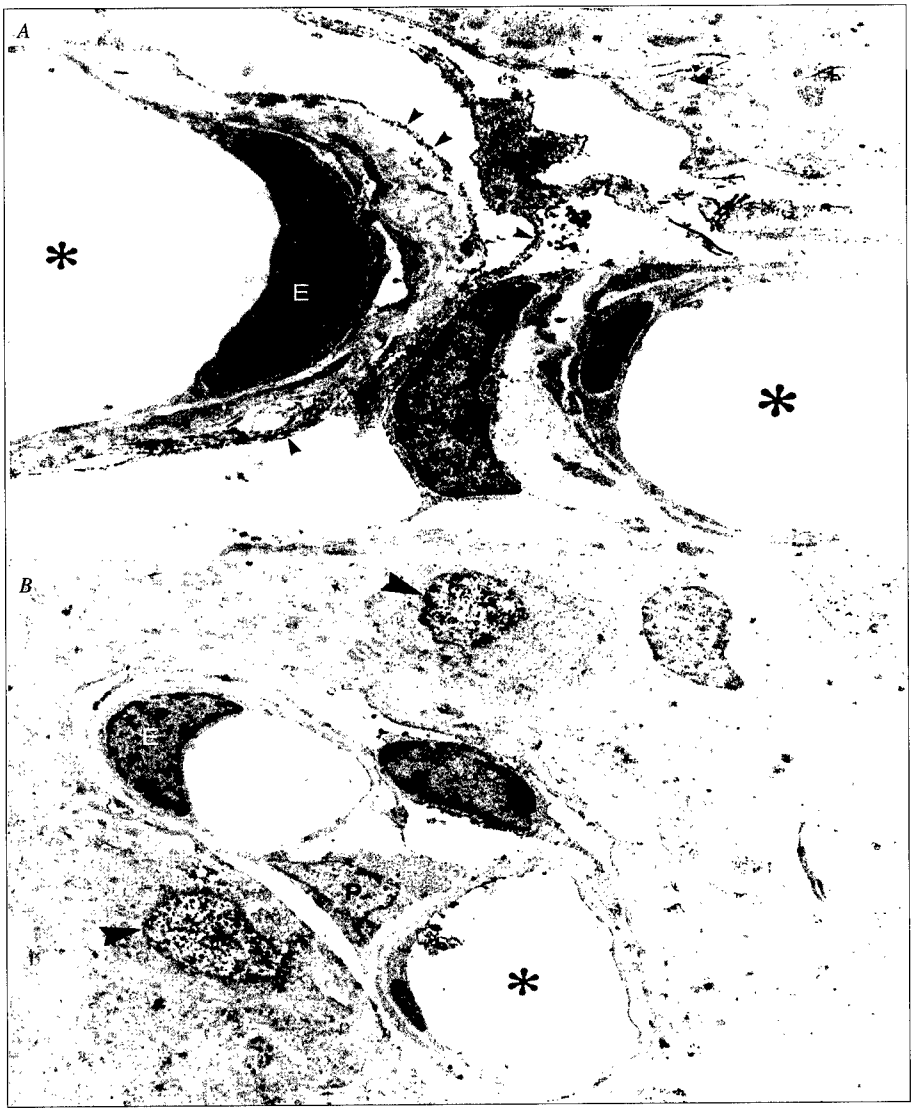

Figure 3. Electron micrographs of sections processed for Fos immunocytochemistry taken from the area postrema of a ferret that had received cisplatin 10 mg/kg i.p.

A: This demonstrates the typical appearance of capillaries and the large perivascular spaces seen in the area postrema. E, endothelial cell nucleus; asterisks indicate the capillary lumen. The arrowheads indicate non-specific immunochemical reaction product lining the perivascular spaces.

B: This is a lower power view of capillaries and accompanying pericyte (P). The arrowheads indicate two Fos-positive neuronal nuclei. The Fos reaction product is seen as a granular deposit.

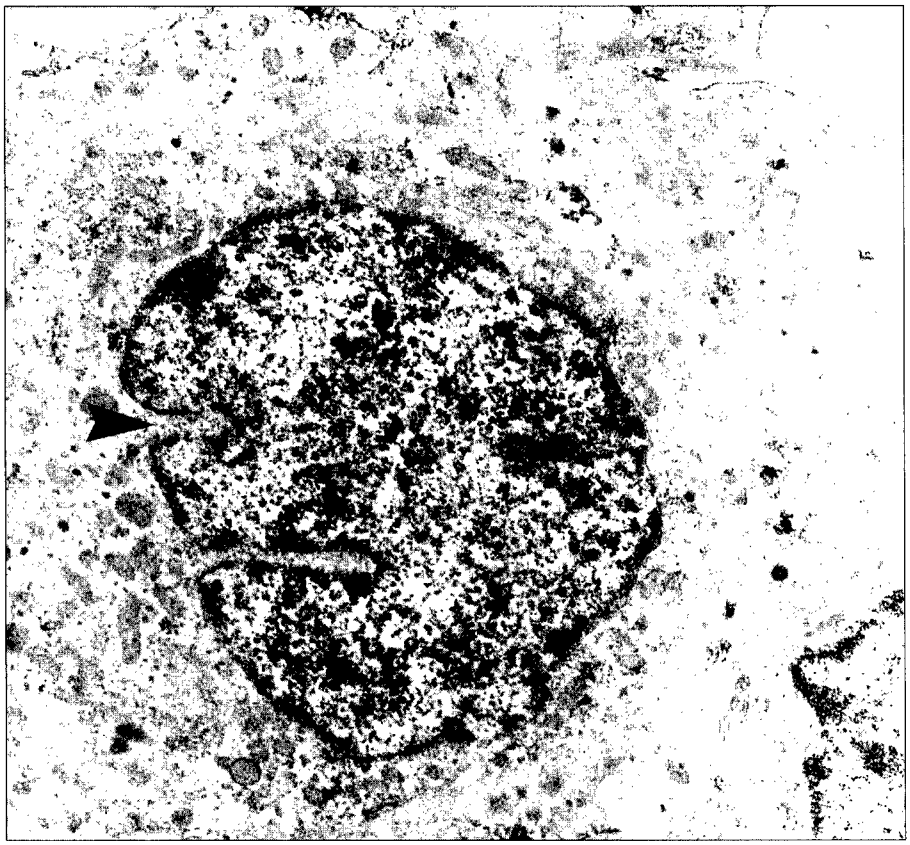

Figure 4. Higher power view of a neuronal nucleus with dense reaction product indicating Fos immunoreactivity. The nucleus has the typical indentations seen in neurones in the area postrema and subnucleus gelatinosus.

EVIDENCE FOR A 'CENTRAL' SITE OF ACTION OF 5-HT_3 RECEPTOR ANTAGONISTS

The 5-HT_3 receptor antagonist, zacopride, inhibits emesis caused by cisplatin when it is given either intracerebroventricularly. or intravenously, and irrespective of the route of administration of cisplatin (30). This is a more interesting observation, and suggests that intracerebroventricular cisplatin does cause emesis through a mechanism that involves 5-HT_3 receptors, and presumably those receptors are located in the dorsal vagal complex. In a subsequent study (33), Higgins and colleagues showed that emesis resulting from intraperitoneal cisplatin in the ferret was inhibited by injections of 5-HT_3 receptor antagonists into the caudal medulla oblongata. This study has often been quoted as confirmation that 5-HT_3 receptor

antagonists exert their anti-emetic actions at the level of the area postrema. Similar effects have been demonstrated when a 5-HT_3 receptor antagonist is injected into the fourth ventricle rather than the medulla oblongata itself (34). However, whereas these studies demonstrate that 5-HT_3 receptor antagonists can exert an anti-emetic effect when given centrally, they do not provide conclusive evidence that this is the site at which they act when given peripherally.

Recent evidence indicates that cisplatin-induced emesis in dogs can be inhibited by intravenous administration of 5-HT_3 receptor antagonists that can cross the blood-brain barrier, but not by one (a quaternized derivative of zatosetron) that cannot (35). However, when quaternized zatosetron is given intracerebroventricularly it does inhibit emesis. This is in contrast to the data obtained by the same authors in ferrets using quaternized tropisetron or zatosetron, which were able to inhibit cisplatin-induced emesis when given intravenously. The reasons for this discrepancy are not clear. It is possible that peripheral 5-HT_3 receptors play a greater role in the control of emesis in the ferret than in the dog. Alternatively, it is known that in the rat at least, neurones within the NTS and dorsal motor nucleus of the vagus have dendrites that extend to the ventricular surface of the fourth ventricle (36), and hence these neurones may be influenced by compounds that do not cross the blood-brain barrier. Whether or not there is a significant difference between ferrets and dogs in the ability of quaternized compounds or their metabolites to cross the blood-brain barrier is also unknown.

WHAT IS THE ROLE OF 5-HT_3 RECEPTORS IN THE BRAINSTEM?

5-HT_3 receptors are present in the dorsal vagal complex by virtue of their presynaptic location on vagal afferent terminals. Activation of 5-HT_3 receptors has only ever been shown to produce depolarization, that is, they are excitatory. 5-HT or 5-HT_3 receptor agonists have been shown to modulate the release of a variety of neurotransmitters, including dopamine (37), cholecystokinin (38), GABA (39), excitatory amino acids (40) and 5-HT itself (41).

There are interesting problems when attempting to understand the action of presynaptic excitatory receptors. Agonists activating such receptors may have complex effects. At low doses, or by analogy at low endogenous ligand activity, agonists will elicit neurotransmitter release as a direct consequence of terminal depolarization. However, this depolarization will, in turn, inhibit release of neurotransmitter evoked by afferent firing, and hence to some extent, the effect of an agonist on a terminal excitatory receptor will depend on the 'tone' of the afferent fibre (42). Added to this is the uncertainty regarding the source of 5-HT that activates the terminal 5-HT_3 receptors. Apart from in rodents and lagomorphs, there is no good evidence that 5-HT neurones exist within the area postrema or its immediate vicinity. Some authors have identified masked indoleamine cells in the area postrema in the rat (43), but we have been unable to identify cells with

immunoreactivity to either 5-HT or tryptophan hydroxylase in human or ferret area postrema (Reynolds and Chen, unpublished data). A small number (about 5%) of vagal afferents in the rat and cat are serotonergic (44, 45) and it is possible that 5-HT released from the central terminals of vagal neurones acts by way of positive feedback to enhance further neurotransmitter release and so to amplify afferent signalling. Unilateral injection of the serotonergic neurotoxin 5,7-DHT into the ferret nodose ganglion reduces 5-HT_3 receptor binding in the ipsilateral dorsal vagal complex by up to 50% in its more rostral part (Reynolds and Leslie, unpublished data), which suggests that some 5-HT_3 receptors may be present on serotonergic vagal afferents. It is of interest, therefore, that the concentration of 5-HT in the dorsal vagal complex is increased in ferrets treated with cisplatin or cyclophosphamide, and that this increase is inhibited by vagotomy or ondansetron (see Chapter 5).

The majority of 5-HT fibres in the dorsal vagal complex emanate from the nearby raphé nuclei (46–48), and in particular from the nucleus raphé obscurus. In immunocytochemical studies, serotonergic fibres and terminals are seen principally in the area postrema and the region immediately subjacent to it, that is, the subnucleus gelatinosus (49–51). Electrical stimulation of the nucleus raphé obscurus induces 5-HT release in the dorsal vagal complex, as demonstrated by *in vivo* microdialysis (52).

Does this imply, therefore, that cisplatin (or other cytotoxic drugs) has an effect on the activity of raphé neurones, which hitherto have not entered into the proposed hypotheses of cytotoxic drug-induced emesis? It is possible that cisplatin directly alters the activity of neurones within the area postrema (in keeping with the Fos immunocytochemical findings), and it is already known that cisplatin can exert direct effects on neurones both *in vivo* and *in vitro* (53, 54). Neurones within the area postrema project almost exclusively to the NTS and parabrachial nucleus (55), and through the NTS projections may influence 5-HT release within the NTS from terminals of fibres that originate in the raphé nuclei (Figure 5). 5-HT so released could then activate 5-HT_3 receptors located on vagal afferent terminals, resulting in release of neurotransmitters (such as glutamate, 5-HT or substance P) and hence the initiation of emesis. The anti-emetic effects of 5-HT_{1A} receptor agonists against cytotoxin-induced emesis may also be relevant here, as activation of presynaptic 5-HT_{1A} receptors will inhibit the release of 5-HT from the terminals of raphé neurones ((56); see also Chapter 23 for a fuller discussion of the possible sites of action of 5-HT_{1A} receptor agonists in other circumstances).

Such a scheme is not in conflict with any of the available data. In particular, the effect of vagotomy in abolishing cisplatin-induced emesis may occur simply because the procedure results in a loss of 5-HT_3 receptors in the dorsal vagal complex, as shown by autoradiography (19). Although one may thus not need to postulate an anti-emetic action of 5-HT_3 receptor antagonists peripheral to the central terminals of the vagus, the presence of 5-HT_3 receptors along the whole gut–vagus–brainstem axis means that circulating 5-HT_3 receptor antagonists almost certainly exert effects at

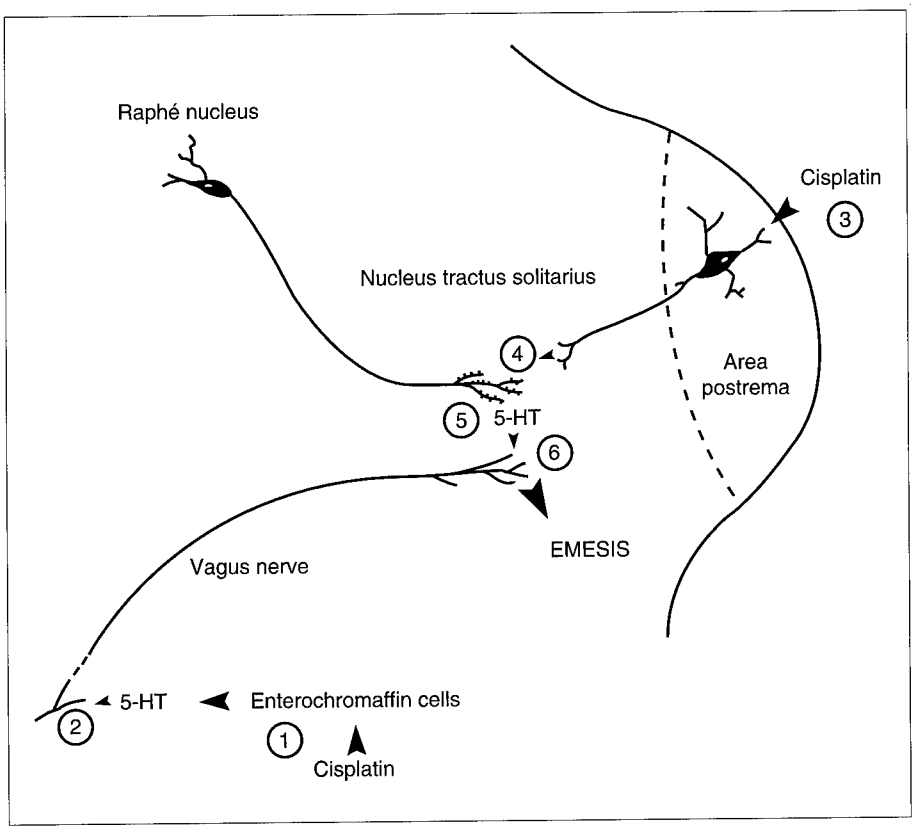

Figure 5. A schematic representation of the proposed events in the dorsal vagal complex following administration of cisplatin. Cisplatin induces release of 5-HT from gut enterochromaffin cells (step 1), which then interacts with 5-HT_3 receptors on peripheral terminals of pseudounipolar vagal afferent fibres (step 2) resulting in vagal afferent firing, which may result in emesis or may act to prime the central pathways for a subsequent action of cisplatin at the area postrema (steps 3–6). Cisplatin also activates neurones within the area postrema (step 3), which project to the nucleus tractus solitarius and cause local release of 5-HT from terminals emanating from the raphé nuclei (step 4). This 5-HT interacts with 5-HT_3 receptors located on vagal afferent terminals in the subnucleus gelatinosus (step 5) and results in release of neurotransmitter from vagal terminals (step 6), ultimately leading to emesis. The neurotransmitters at steps 4 and 6 are unknown, but candidates include glutamate, substance P or opioids. It is clear from this scheme that vagotomy may inhibit cisplatin-induced emesis because the lesion results in a loss of the 5-HT_3 receptors involved at step 5, and the priming events in step 2 cannot occur. Furthermore, removal of the area postrema will destroy the neurones that trigger 5-HT release from raphé terminals and hence cisplatin will not cause emesis, but emesis can still be elicited by vagal afferent electrical stimulation, as this pathway is still intact (see Chapter 2).

more than one site. This is discussed in detail in the preceeding chapters. The results of acute vagotomy as reported by Andrews and colleagues (2) suggest that in radiation-induced emesis at least, vagal afferent firing is the driving force for emesis.

The preceeding chapters have focussed on the peripheral effects of 5-HT_3 receptor antagonists, and I have dealt more with the central terminals of the vagus nerve, which hitherto have been largely ignored. The most logical conclusion from the available information is that 5-HT_3 receptor antagonists exert anti-emetic effects both centrally and peripherally, but the key to their site of action is the vagus nerve. In some circumstances, it is possible that effects at the central terminals of the vagus are more important than at their peripheral end, and in others, peripheral effects may predominate. It is likely that cisplatin-evoked vagal afferent firing results in priming of central emetic pattern generators, such that any stimulation that then occurs at the level of the area postrema elicits emesis more readily. Similarly, if area postrema neurones are stimulated by a circulating cytotoxic drug, this may lower the threshold to emesis driven by primarily visceral afferent activity.

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Chapter 12

5-HT_3 receptor antagonists and radiation-induced emesis: preclinical data

R. K. Harding

INTRODUCTION

Ten years ago, radiation-induced emesis in animals was incompletely controlled by a baffling polypharmacy including; dopamine and histamine (H_1) receptor antagonists, steroids, anti-inflammatories, opioid-receptor antagonists and other drugs (1). Combination anti-emetic therapy, led usually by a D_2 receptor antagonist, or single therapy with a D_2 receptor antagonist, was typically employed for radiotherapy patients. Our concept of the mechanism of emesis was dominated by the model proposed by Wang and Borison (2). Over the years, this model had become interpreted rather selectively to support a pivotal role for the chemoreceptor trigger zone, located in the area postrema, in emesis induced by radiation and other cytostatic treatments. Apomorphine was the standard experimental emetic; therefore the dopamine receptor was assumed to be the key receptor to block. Domperidone was the new anti-dopamine compound that was designed to be a poor penetrator of the blood-brain barrier, thus resulting in fewer extra-pyramidal side-effects, while gaining access to the more permeable area postrema. This, it was reasoned, would be the best way to counteract the unidentified humoral factor(s) purported to cause the emesis seen following exposure to ionizing radiation. The dog was the predominant animal model for the study of radiation-induced vomiting, followed by the non-human primate and the cat. Everything changed with 5-HT_3 receptor antagonists.

5-HT_3 RECEPTOR ANTAGONISTS IN ANIMAL STUDIES

Prevention of radiation-induced vomiting

The first published recognition that 5-HT_3 receptor antagonists might have a role in the control of emesis stimulated by cytostatic treatments appeared in 1986 (3). Many reports have appeared since. The vast majority have been for chemotherapy compounds and other chemical emetics. Table 1 summarizes the results from experiments in irradiated animals, designed to investigate the anti-emetic potential of 5-HT_3 receptor antagonists. There are multiple datasets in ferrets for both granisetron and ondansetron, with the former being the more thoroughly researched. The consistent observation among all of these experiments was the complete

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inhibition of emesis in all, or most animals. Typically, in those experiments where emesis did persist in a minor proportion of the animals, there was a significant increase in the latency to the first episode, and a significant decrease in the numbers of episodes, often to a single event in each responder. These results proved the superiority of 5-HT_3 receptor antagonist anti-emetics over any other therapy, in the prevention of radiation-induced vomiting.

Work in the ferret has shown potent (microgram range) anti-emetic effectiveness, regardless of the route of administration (intravenous intramuscular subcutaneous or per os). Studies with granisetron and ondansetron in ferrets fitted with chronic indwelling intra-jugular catheters, have shown that a bolus injection of the antagonist can stop established emesis immediately upon injection. This result, which cannot be equalled by anti-emetics inactive at 5-HT_3 receptors, implies that the emetic mechanism relies upon a continual release of stimulus. These data in ferrets also predicted the result in radiotherapy patients (see Chapter 13), where intravenous bolus injection of ondansetron or granisetron proved to be successful rescue medications.

Insights into the mechanisms of radiation-induced vomiting

A careful series of experiments in the irradiated ferret conducted by Andrews and his collaborators (4), has shown the critical role of the afferent vagus in the conduction of the emetic stimulus, from the viscera to the coordinating region of the medulla responsible for initiating the emetic response. Andrews has proposed that the anti-emetic action of 5-HT_3 receptor antagonists is consistent with a chemical vagotomy. The work by Andrews *et al.* has caused a re-evaluation of the model proposed by Wang and Borison (2). To be fair, the model and Borison's work, always provided a role for the vagus, but this feature was largely ignored. It has been experiments in the ferret and the availability of 5-HT_3 receptor antagonists, which have provided the means to clarify the issues, and have caused a shift in the interpretation of the Wang and Borison model.

Table 1. The following 5-HT_3 receptor antagonists inhibit radiation-induced emesis.

Drug	Species
Metoclopramide (high dose)	Ferret
Renzapride	Ferret
Ondansetron	Ferret
Granisetron	Dog
	Ferret
Batanopride	Ferret
Zacopride	Ferret
Y 25130	Ferret
Tropisetron	*Suncus*

References for the data may be found in (8), (11), (13) and (14). Renzapride (BRL 24924), ondansetron (GR 38032F), granisetron, (BRL 43694), batanopride (BMY 25901), tropisetron (ICS 205-930).

RADIATION EFFECTS AND SYMPTOMOLOGY

Radiation: another emetic 'toxin'

Davis *et al.* (5) have proposed that the vomiting response may be viewed as an hierarchical organization of defences, designed to protect the body from damage by ingested toxins. Chemo- and mechanoreceptors located in the viscera are key components of this design. The 5-HT_3 receptor antagonists have provided the pharmacological means to characterize this visceral mechanism. The acute vomiting response that closely follows exposure to ionizing radiation is a stereotypic response to signals and effects that are interpreted by the body as being caused by an 'ingested toxin'.

Radiation has been a valuable tool, and its use in future studies of the mechanisms of emesis and prevention by anti-emetics should not be overlooked. Unlike chemical emetics, radiation may be applied to specific regions or sites. This feature has been important in identifying the upper abdomen of animal models and of patients, as the most sensitive emetic volume. The 'pharmacology' of radiation has been very useful. The deposition of radiation energy is instantaneous; therefore there are no kinetics of absorption or washout, and no confounding variables concerning species-specific capacities to metabolize or convert chemical emetics.

Actions of radiation

In all animals with an emetic response to radiation, the latency period seen before the onset of emesis implies that emesis is not induced directly by radiation exposure. Some second-order mechanisms are necessary following the deposition of radiation in tissues. Release of 5-HT from enterochromaffin-like cells located in the bowel mucosa (see Chapter 7), is a likely arbiter of the emetic process. It is not clear how radiation or other emetics accomplish this release. We do know that free radicals are released into tissues and cells immediately following irradiation, and that in *Suncus murinus* free-radical scavengers have some role as anti-emetics. An inflammatory reaction has been documented at 2 hours in the rat (6), and our further studies have demonstrated an inflammatory reaction in the ferret bowel. This may occur too late to contribute to the emesis seen in ferrets, but it may be important in other species.

Typically, it is the lethal cytotoxic effects of radiation that are studied and cited as the major explanation for the symptomology. However, emesis may be induced by sub-lethal doses of radiation. The emetic dose of radiation is well below that which would lead to appreciable somatic cell death, and emesis occurs well before failure at mitosis accounts for the death of actively dividing cell types. It is very clear that acute radiation-induced vomiting is caused by pathophysiological, and not histopathological factors; at least as far as widespread frank damage is concerned. However, I believe that apoptosis, or active/inducible cell death, may be a potentially important contributor. Potten (7) has shown that there is a sensitive sub-population of stem cells in the crypts, which undergo morphologically distinct stages of cell death that are noticeable in rodents from 30 minutes, and complete by

3 hours post-irradiation. In summary, apoptosis is very radiation-sensitive, rapid, and is maximal at low doses (100 cGy). The sensitive crypt cells that undergo apoptosis immediately following irradiation, and some of the equally sensitive intra-epithelial lymphocytes, are located proximal to the enterochromaffin-like cells. Could these dying cells contribute to the stimulus for 5-HT release?

Importance of the ferret

It is clear from Table 1 that the ferret is the predominant animal model used in the characterization of 5-HT_3 receptor antagonist anti-emetics. Together, this new animal model and 5-HT_3 receptor antagonist anti-emetics, have been crucial in establishing the importance of visceral afferents and serotonin stimulation in the initiation of emesis by radiation and other cytostatic treatments. But do the effects of radiation and of 5-HT_3 receptor antagonist anti-emetics seen in the ferret mimic those seen in patients? The answer is largely yes but there are some differences worthy of note. The following discussion highlights some of the similarities and differences between humans and ferrets, and ferrets and other animal models.

Acute post-radiation symptoms in ferrets and other animals

Most experiments with 5-HT_3 receptor antagonist anti-emetics in irradiated ferrets show complete inhibition of vomiting. However, our data in the beagle dog and the results in patients (8), show that breakthrough vomiting does occur in a minority of subjects. These emetic responses are characterized by long latency and single or few vomiting responses per subject. One of the other species-specific differences in the responses to radiation is the incidence of diarrhoea. Radiation-induced diarrhoea in the ferret is present at doses below the ED_{50} for vomiting. A prompt dose of 400 cGy results in a maximal and profound diarrhoea response in ferrets. This latter dose is barely threshold for radiation-induced diarrhoea in the dog and in humans.

Table 2 summarizes the emetic sensitivity of the laboratory species known to exhibit radiation-induced vomiting, and compares the data to those reported in humans (8, 9). As can be seen, the ferret is most sensitive and exhibits an ED_{100} at a dose that is barely threshold for the next most sensitive species, humans. There is no ED_{100} in humans. Table 3 compares radiation sensitivities for emesis and lethality in the four most relevant species. Note that the ED_{50} and the LD_{50} are similar for the dog and for humans. The LD_{50} in the ferret, at less than 200 cGy, is anomalous and lower than that, for virtually any animal tested (10). The dose–response curve for radiation-induced lethality is very steep. The LD_{50} dose reported here in the ferret may be as little as an LD_{10} in humans. Therefore, the ferret is much more sensitive to the effects of ionizing irradiation than humans. Taking the data from Table 3, we can construct a continuum from lowest to highest sensitivity for both the emetic and the lethal effects of irradiation, which shows the dog to be most like humans:

rhesus monkey > dog = human > ferret

Table 2. Ionizing radiation: emetic sensitivity and species comparisons.

Species	Dose (cGy)		
	ED_{10}	ED_{50}	ED_{100}
Human	< 100	200	400 (ED_{80})
Dog	–	230	700
Ferret	75	100	125
Monkey	–	450	800 (ED_{80})
Cat	–	> 2000	> 4000
Suncus	–	429	800

ED_{10}, ED_{50}, ED_{80}, ED_{100}, dose of radiation required to cause emesis in 10%, 50%, 80% and 100% of the subject population, respectively.

Table 3. Ionizing radiation: emetic sensitivity, latency and LD_{50}.

Species	Latency (minutes)	Dose (cGy)	
		ED_{50}	LD_{50}
Human	60–90	200	290
Dog	80–90	230	260
Ferret	30	100	< 200
Rhesus monkey	45	450	550

Latency, time to the first emetic episode; ED_{50}, dose of radiation required to cause emesis in 50% of the subject population; LD_{50}, dose of radiation required to kill 50% of the subject population. In humans, the value is reported as a $LD_{50/60}$, the dose calculated to kill 50% of the subjects within 60 days. In animals, the assessment period is 30 days and the value reported as $LD_{50/30}$.

Although this may simply be a coincidence, these data, taken together with observations concerning symptomatology and coverage by 5-HT_3 receptor antagonist anti-emetics, provide a case for the contention that the dog, in this instance, may be the most faithful animal model for humans.

ANIMAL MODELS: WHAT CAN WE LEARN BEYOND EFFICACY?

It is clear that the ferret has been a valuable predictor of the anti-emetic efficacy of 5-HT_3 receptor antagonists. It can be argued that differences in the absolute quantity of emetic stimulus (radiation dose required in ferret and human) is irrelevant, so long as the same emetic mechanisms are invoked and no additional pathways or effects are stimulated. Differences in latency provide one indication of a potentially significant mechanistic difference. Most emetic episodes are finished in the ferret at

the time that vomiting is just beginning in humans (dose of radiation ranging from ED_{50} to maximal emetic dose). In the ferret, the maximal emetic dose of 400 cGy results in emesis in all animals beginning at around 16 minutes (11). Only very large doses in humans (several tens of Gy), likely to be associated with cerebral oedema, can result in emesis in less than 30 minutes (9). Either the emetic mechanism in the ferret is more directly stimulated by radiation, or the 'sum' of emetic stimuli necessary to induce the response in humans is slower to accumulate.

The discrepancy between the near perfect anti-emetic efficacy of 5-HT_3 receptor antagonists in irradiated ferrets and the incomplete blockade in radiotherapy patients, has been ascribed to additional emetic stimuli resulting from the malignant disease being treated (4). Although this may be a plausible explanation it cannot account for the breakthrough emesis that is seen in irradiated dogs. Interestingly, the dog provides an additional opportunity to test the hypothesis. Veterinary oncology is a small but active practice in canine medicine. It should be possible to compare the anti-emetic efficacy of a particular 5-HT_3 receptor antagonist in normal dogs exposed to a therapeutic dose of ionizing radiation, with that in dogs receiving radiotherapy for a carcinoma.

CONCLUSIONS

The evidence for the plasticity of the emetic response has been reviewed by Andrews and Davis (4). Their contention (based on ablation studies and work with 5-HT_3 receptor antagonists) is that as one emetic mechanism or pathway is blocked, other routes are uncovered or accentuated to provide emetic protection. Harding (12) described predominant and parallel emetic mechanisms, present in the same animal and exhibiting differential responsiveness to emetic stimuli. The demonstration of plasticity is further proof of the existence of parallel pathways and of the preferential use (unique to a species or an emetic) of a predominant emetic pathway. For radiation-induced emesis and blockade by 5-HT_3 receptor antagonists, this may mean that in the ferret, the visceral route has near sole responsibility for the initiation of the emetic signals. However, in the irradiated dog or human, although the visceral route predominates, there are contributions to the emetic response that may not be viscerally mediated and/or are incompletely blocked by 5-HT_3 receptor antagonists.

Ionizing radiation is a valuable tool to study these contentions and to conduct selective experiments to further dissect the emetic response. We shall continue to need animal models to help us to address other symptoms such as diarrhoea and delayed emesis, which have resisted 5-HT_3 receptor antagonist therapy. We should be mindful of what the animals are telling us, and be alert to their virtues and to their limitations.

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Chapter 13

Clinical evidence for 5-HT_3 receptor antagonist efficacy in radiation-induced emesis

T. R. Spitzer

INTRODUCTION

Nausea and vomiting are common complications of radiation therapy, particularly when the treatment involves the upper abdomen (1–3). Factors influencing the incidence and severity of radiation therapy-induced emesis include size and location of the treatment field, dose and dose rate, prior chemotherapy, and possibly age (1–4). An acute radiation sickness, characterized by the sudden onset of vomiting (with or without antecedent nausea) has been well documented among survivors of atomic bombs, accidentally exposed individuals and patients who receive total-body irradiation (TBI) as preparative therapy for bone marrow transplantation (3–6). Among patients receiving half-body irradiation, radiation-induced emesis is most severe for those who receive upper hemibody irradiation (2). High dose rates, at least for patients receiving TBI, are likely to be associated with an earlier onset of nausea and vomiting.

Although the aetiology of irradiation-induced emesis is probably multifactorial, a compelling body of experimental evidence suggests that serotonin, which is released from enterochromaffin cells of the intestine following irradiation, leads to activation of peripheral and/or central 5-HT_3 receptors (7). Clinical evidence for the importance of the 5-HT_3 receptor mechanism of irradiation-induced emesis derives from several trials in which selected 5-HT_3 receptor antagonists have been evaluated. Both ondansetron and granisetron have been shown to be highly effective in the prevention of irradiation-induced emesis following hemibody and whole-body irradiation (9–18). The purpose of this review is to define the natural history of irradiation-induced emesis and to review the clinical evidence for an impact of 5-HT_3 receptor antagonists on its incidence and severity.

IRRADIATION-INDUCED EMESIS: NATURAL HISTORY

The natural history of irradiation-induced emesis is difficult to define because of multiple and confounding variables, including concomitant (or antecedent) chemotherapy, variable use of anti-emetic regimens, different irradiation sources,

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and variable reporting practices (1–4). Nonetheless, data regarding the emetic patterns following hemibody and whole-body irradiation, which represent the most potent irradiation-induced emetic stimuli, have been published and will be reviewed.

Danjoux and colleagues followed 189 patients who received 100–1000 cGy at 20–60 cGy/minute of single-dose half-body irradiation (2). Radiation sickness occurred most commonly among patients who received irradiation to the upper half of their bodies (73 out of 88 patients; 83%) compared with 39 out of 101 (39%) of patients who received irradiation to the lower half of their bodies. Patients typically experienced sudden nausea and vomiting within 90 minutes of treatment, and had an average of five emetic episodes. Premedication with several different anti-emetic drugs (or combination anti-emetic regimens) had no apparent effect on the incidence of vomiting. Salazar *et al.* employed half-body irradiation therapy in 40 patients with advanced malignancies (1). A standard treatment regimen of 800 cGy delivered at 34 cGy/minute via a 10 MeV linear accelerator was administered. Most of the 20 evaluable patients who did not receive anti-emetic premedication experienced nausea or vomiting, whereas only 4 out of 12 (33%) and 2 out of 12 (17%) of patients receiving lower-half-body irradiation experienced nausea or vomiting, respectively. Six patients who received a combination anti-emetic regimen that included corticosteroids had minimal acute symptoms.

Nausea and vomiting following TBI has historically been a universal problem (3, 4). Westbrook *et al.* studied the emetic response of 305 patients who received TBI as preparative therapy for bone marrow transplantation (3). All patients received chemotherapy (either with cyclophosphamide or melphalan) immediately before irradiation, then received 9.5–11.5 cGy at 0.02–0.1 cGy/minute as a single treatment. In total, 117 of the patients received anti-emetics, as required for nausea and vomiting. Subsequent patients received hydrocortisone and phenobarbitone before irradiation, then on a regular (2–4 hourly) basis following irradiation. Vomiting did not occur until a cumulative dose of 2.0–3.0 cGy had been administered. Recurrent vomiting frequently occurred after 2–5 days. A peak incidence of vomiting occurred 4 days after irradiation. Factors that appeared to influence the incidence of vomiting were radiation source (with increased vomiting following irradiation from the single-source ^{60}Co unit), and age (with significantly less vomiting in patients under 10 years old).

A retrospective review of 29 patients at Georgetown University Medical Center who received 13.2 cGy of TBI at 0.22 cGy/minute in 11 fractions over 4 days prior to chemotherapy with cyclophosphamide (with or without etoposide) showed that vomiting was universal, despite aggressive combination anti-emetic therapy (4). The median number of emetic episodes was five (range 1–32), with the peak number of emetic episodes occurring on the first day of irradiation (Figure 1). Nausea and vomiting were commonly seen after the first fraction of 1.2 cGy, contrary to the experience of Westbrook.

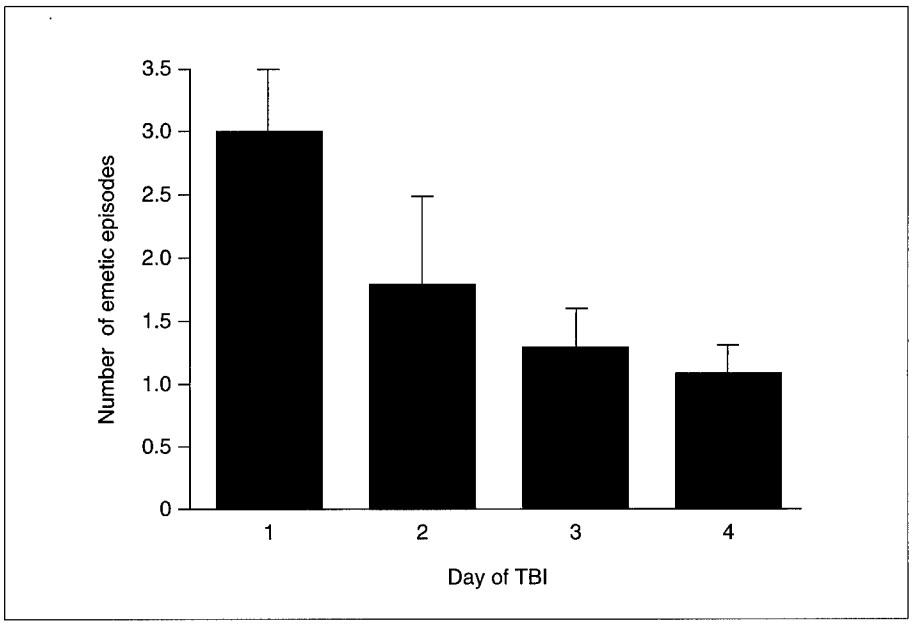

Figure 1. Number of emetic episodes ± SEM according to treatment day for 29 patients receiving TBI at 13.2 cGy in 11 fractions.

ANTI-EMETIC TRIALS WITH 5-HT_3 RECEPTOR ANTAGONISTS

Based on the experimental evidence demonstrating effective prevention of irradiation-induced emesis by 5-HT_3 receptor antagonists, and the extensive clinical experience demonstrating the safety and efficacy of 5-HT_3 receptor antagonists in the prevention of chemotherapy-induced emesis, multiple trials have been undertaken in an attempt to define the anti-emetic properties of the 5-HT_3 receptor antagonists in the prevention of irradiation-induced emesis. Several of these trials involved high dose single or fractionated upper abdominal radiation therapy. Priestman and colleagues conducted a randomized, double-blind evaluation of ondansetron and metoclopramide following single-dose irradiation therapy to the upper abdomen (9). In all, 92% of patients who received ondansetron had complete emetic control during the first 24 hours after therapy compared with only 46% of patients receiving metoclopramide (p = 0.001). Control of nausea was also significantly greater among the ondansetron-treated patients. In a prospective, randomized, double-blind trial conducted by Priestman *et al.,* in which ondansetron was compared with prochlorperazine for prevention of emesis following fractionated (five daily treatments) irradiation to the upper abdomen, ondansetron was also found to provide significantly greater anti-emetic protection than the control group (16). The percentage of patients showing a complete response during their treatment

Table 1. 5-HT_3 receptor antagonist trials for TBI-induced emesis.

Centre	Trial design	No. of patients	Conditioning regimen	Drug	Dose	Outcome	Reference
Hospital for Sick Children, Bristol, UK	RET	15	CY 120 mg/kg → TBI 1.8 cGy b.d. at 0.2 cGy/min × 4 days	OND	5 mg/m^2 i.v. q8h × 7 days	93% CR or MR	(10)
Royal Free, London	RET	32	TBI 7.5 cGy at 0.5 ± 0.02 cGy/min	GRA	40 μg/kg prior to TBI	97% CR or MR	(11)
Universität Kinderklinik, Münster, BRD	RET	15	NS	OND	2, 4 or 8 mg i.v. t.d.s.	57% CR (vomiting) 86% CR or MR (nausea) on 'worst' TBI day	(14)
Berlin	RET	25	TBI 2.0 cGy b.d. or 4.0 cGy q.d.s. × 3 days	OND	5 mg/m^2 before TBI (additional 2 doses as required)	88% CR or MR	(17)
Royal Marsden, London	PRO	20	MEL 110 mg/m^2 TBI 10.5 cGy at 0.04 cGy/min	Phenobarbitone + corticosteroids ± OND	8 mg i.v. before TBI	Fewer emetic episodes ($p = 0.029$) compared with placebo	(15)
Georgetown, Washington, DC	PRO	20	TBI 13.2 cGy at 0.22 cGy/min in 11 fractions over 4 days	OND (*versus* placebo)	8 mg p.o. before each TBI dose	Fewer emetic episodes ($p = 0.005$) Delayed time of onset ($p = 0.003$) compared with placebo.	(18)

RET, retrospective; PRO, prospective; CY, cyclophosphamide; OND, ondansetron; GRA, granisetron; TBI, total-body irradiation; CR, complete response; MR, major response; MEL, melphalan; NS, not specified.

course (61% *versus* 35%; $p = 0.002$) and the percentage of emesis-free days was significantly greater in the ondansetron-treated group.

Henriksson and colleagues treated 33 consecutive patients receiving fractionated upper-abdominal irradiation to a field of 100 cm^2 at a daily dose of 1.8–4.0 cGy, with oral ondansetron, 8 mg three times daily (13). Complete anti-emetic protection was observed in 26 out of 33 (79%) patients, encompassing 94% of their treatment days.

Several retrospective series and two prospective, double-blind, placebo-controlled, randomized trials have evaluated the anti-emetic efficacy of 5-HT_3 receptor antagonists in the prevention of TBI-induced emesis (Table 1). Hewitt and colleagues described 15 children who received TBI at a total dose of 14.4 cGy (1.8 cGy twice daily for 4 days) following high dose cyclophosphamide (10). Eleven patients had fewer than five emetic events in the 'worst 24 hour period' with six of the patients experiencing no vomiting at all. Of a total of 100 'ondansetron patient days', 83 were without emetic episodes (vomiting or retching).

Jurgens and McQuade treated 15 children undergoing bone marrow transplantation with ondansetron, for prevention of emesis caused by a combination of chemotherapy and radiotherapy (14). The ondansetron dose was 2, 4 or 8 mg (depending on body surface area) three times daily during chemotherapy and TBI. Complete protection from vomiting on the 'worst' TBI day was achieved in 57% of the patients. Complete and 'major' protection from nausea was seen in 86% of the patients.

Hunter *et al.* evaluated granisetron for the prevention of TBI-induced emesis in 32 consecutive bone marrow transplant patients (11). Patients received TBI in a total dose of 7.5 cGy at a dose rate of 0.15 ± 0.02 cGy/minute. A single dose of granisetron of 40 μg/kg was given over 5 minutes, 30–60 minutes before irradiation. No chemotherapy was administered prior to TBI. Of 32 patients, 31 (97%) achieved either total protection from emesis (18 patients; 56.3%) or had a 'major' response (13 patients; 40.6%).

In an open, non-randomized, pilot study, Logue and colleagues evaluated the efficacy of two intravenous granisetron doses (20 μg/kg and 40 μg/kg) before a single midline irradiation dose of 8 cGy to the lower half of the body (12). Complete responses were observed in nine out of 13, and six out of nine patients in the low and high dose levels, respectively.

Schwella and colleagues treated 25 patients with ondansetron for prevention of emesis following fractionated TBI (17). TBI was delivered either on three consecutive days at 2.0 cGy twice daily to a total dose of 12.0 cGy (for patients with acute lymphoblastic leukaemia), or a single fraction of 4.0 cGy/day for 3 days (total dose 12.0 cGy) for patients with acute and chronic myeloid leukaemia. Ondansetron was given at a dose of 5 mg/m^2 1 hour before TBI, and on two further occasions if required. In addition, 19 of the 25 patients received levomepromazine by continuous infusion during TBI. Most (22) of the 25 patients had fewer than three emetic episodes during TBI, with 11 of the 25 (44%) achieving complete protection. A total of 52 of the 75 patient days (69%) were associated with no emetic episodes.

Tiley and colleagues conducted a prospective, double-blind, placebo-controlled trial, in which the efficacy of a single 8 mg intravenous dose of ondansetron was

evaluated (15). Twenty patients, who received melphalan, 110 mg/m^2, the day before a single-dose TBI at 10.5 cGy (0.04 cGy/minute) were randomized to receive ondansetron or placebo before the TBI. All patients also received phenobarbitone and corticosteroids (dexamethasone and hydrocortisone) for emetic control. Significantly fewer patients receiving ondansetron had an emetic event (1 out of 10 *versus* 5 out of 10 who received placebo; $p = 0.029$).

In a prospective, randomized, double-blind, placebo-controlled trial conducted at Georgetown University Medical Center, we evaluated the anti-emetic efficacy of oral ondansetron for TBI-induced emesis (18). Twenty patients, who received TBI at a total dose of 13.2 cGy in 11 fractions over 4 days before cyclophosphamide chemotherapy, were randomized to receive either placebo or ondansetron, 8 mg orally, before each TBI session. Patients who experienced two or more emetic episodes between TBI sessions, or a total of five emetic episodes during the 4 days (representing the median number of emetic episodes in a historical control group receiving multiple non-5-HT_3 receptor antagonist anti-emetic medications), received rescue therapy with intravenous ondansetron at a dose of 0.15 mg/kg. Six out of 10 patients randomized to receive oral ondansetron completed the entire 4-day study without the need for additional anti-emetic therapy, whereas none of 10 patients who received placebo completed the study without rescue therapy (nine of whom required rescue therapy on the first day of TBI). Overall, significantly fewer emetic episodes were experienced by patients who received oral ondansetron than by those receiving placebo ($p = 0.005$). The efficacy of rescue therapy was also demonstrated in this study. Six placebo patients treated with rescue intravenous ondansetron required no further anti-emetic therapy. However, we failed to achieve control of emesis in all five of the ondansetron-treated patients who subsequently required rescue ondansetron therapy, and they were removed from the study.

A pharmacokinetic evaluation of ondansetron, as well as measurements of plasma and urinary 5-hydroxyindole acetic acid (5-HIAA), noradrenaline and dopamine were performed. No relationship was observed between peak ondansetron concentration or the area under the concentration *versus* time curve and the number of emetic episodes, suggesting the possibility that the efficacy of ondansetron is related to factors other than peak drug concentration (for example, drug concentration at the receptor site) (18). Also observed were significantly higher levels of urinary 5-HIAA excretion in the placebo group, suggesting the possibility that ondansetron led, either directly or indirectly, to a decrease in irradiation-induced serotonin synthesis or release (19).

FUTURE DIRECTIONS

The introduction of 5-HT_3 receptor antagonists has resulted in a dramatic improvement in the control of the most emetogenic of cancer therapies, namely TBI. Similarly, these agents have shown marked efficacy in the prevention of nausea and

vomiting associated with other highly emetogenic radiation treatments, such as hemibody irradiation and upper-abdominal irradiation. Despite these advances, several questions remain regarding the anti-emetic efficacy and mechanism of action of these new anti-emetic agents. Among these questions is whether a dose–response relationship exists for the 5-HT_3 receptor antagonists. Although anti-emetic efficacy was demonstrated convincingly in our placebo-controlled trial, protection was incomplete. Four out of 10 patients treated with oral ondansetron (40%) experienced significant nausea and vomiting requiring rescue anti-emetic therapy. Our subsequent experience with higher doses of intravenous ondansetron (0.15 mg/kg i.v. before each TBI session) or granisetron (1 mg i.v. before each TBI session) has shown complete anti-emetic protection in approximately 80% of patients (unpublished observations), suggesting that dose (or perhaps route of administration) may be important in optimal anti-emetic control. The role of combination anti-emetic therapy also requires further investigation. Based on pilot data showing that the combination of ondansetron and dexamethasone given before hemibody irradiation was well tolerated and effective in controlling emesis (20), and a randomized trial showing the superiority of the combination of granisetron and dexamethasone for control of chemotherapy-induced emesis (21), trials comparing a combination of 5-HT_3 receptor antagonists and dexamethasone with 5-HT_3 receptor agents alone for the prevention of TBI-induced emesis are warranted. Although the impact of 5-HT_3 receptor antagonists on delayed nausea and vomiting will be more difficult to evaluate because of the confounding variable of high dose chemotherapy, this too should be addressed. Combination anti-emetic therapy, based on results of the chemotherapy trial in which dexamethasone was important for protection from delayed nausea and vomiting, should be considered. Finally, further definition of the mechanism of action of 5-HT_3 receptor antagonists in the prevention of irradiation-induced emesis will be helpful in optimizing the design of future anti-emetic treatment strategies. Understanding the complex interaction of drugs and their receptors, as well as the impact of the drugs on synthesis or release of important mediators of nausea and vomiting, will probably lead to more effective strategies for the complete prevention of immediate and delayed nausea and vomiting.

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Chapter 14

Clinical evidence for the involvement of serotonin in acute cytotoxic-induced emesis

L. X. Cubeddu, A. B. Alfieri and I. S. Hoffman

INTRODUCTION

Research conducted in the last decade has led to a better understanding of the mechanisms whereby cancer chemotherapeutic drugs induce emesis. The control of nausea and vomiting induced by cytotoxics has also greatly improved. The discovery that selective antagonists of serotonin-3 (5-HT_3) receptors are effective anti-emetics, led to the view that serotonin played a major aetiological role in nausea and vomiting caused by cytotoxic drug treatment. Granisetron, ondansetron and tropisetron are some of the 5-HT_3 receptor antagonists currently approved for clinical use.

In this study, we review the major clinical evidence supporting the involvement of serotonin in emesis induced by treatment with cytotoxic drugs. This evidence may be summarized as follows:

1) the anti-emetic efficacy of selective antagonists of 5-HT_3 receptors;
2) the abolition of cisplatin-induced emesis after pretreatment with para-chlorophenylalanine (PCPA), a serotonin-synthesis inhibitor;
3) increases in plasma and urine concentrations of 5-hydroxyindoleacetic acid (5-HIAA), and in plasma chromogranin A (CgA) concentrations, associated with treatment with cytotoxics. Both 5-HIAA and CgA are markers of serotonin release from enterochromaffin (EC) cells.

ANTI-EMETIC EFFICACY OF 5-HT_3 RECEPTOR ANTAGONISTS

The well demonstrated anti-emetic efficacy of 5-HT_3 receptor antagonists is the most important evidence in favour of the participation of serotonin and 5-HT_3 receptors in emesis induced by treatment with cytotoxics. It should be emphasized, however, that not all cytotoxics elicit emesis through the same mechanism, and that one cytotoxic may induce emesis by more than one mechanism. The anti-emetic action of 5-HT_3 receptor antagonists may be explained by one or more of the following mechanisms (see also Chapter 11).

1) Some cytotoxics induce the release of serotonin from EC cells. This serotonin may act on 5-HT_3 receptors located on visceral afferent fibres, increasing afferent visceral input to the nucleus tractus solitarius. The antagonists may

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exert their anti-emetic action by blocking the effects of serotonin on 5-HT_3 receptors present on visceral afferent fibres.

2) Some cytotoxics may not elicit the release of serotonin from EC cells. The serotonin secreted from EC cells, under basal conditions (tonic release) could act on 5-HT_3 receptors on visceral afferent fibres, sensitizing the emetic reflex. The cytotoxic would act directly or indirectly, for example through the generation of physical and/or chemical stimuli (i.e. free-radical formation; release of inflammatory mediators). These stimuli would act synergistically with serotonin to induce visceral afferent stimulation. 5-HT_3 antagonists would prevent the sensitizing action of serotonin.
3) Activation of centrally located 5-HT_3 receptors (i.e. nucleus tractus solitarius and/or area postrema) is required for cytotoxic-induced emesis to occur (central action). Blockade of this pathway with 5-HT_3 receptor antagonists would prevent emesis.

5-HT_3 receptor antagonists are ineffective against emesis induced by apomorphine, morphine, loperamide, cholecystokinin, motion and cisplatin-induced delayed emesis (1); however, they are effective against acute emesis associated with chemo- and radiation therapy, emesis induced by ipecacuanha, surgery (postoperative period), and acute infections such as gastroenteritis and staphylococcal enterotoxin (2). These results indicate that 5-HT_3 receptor antagonists do not exert a general, non-specific anti-emetic action. Rather, they block specific emetic pathways where visceral afferents are important, and where the release of serotonin plays an important triggering and/or sensitizing action.

SEROTONIN METABOLISM AND RELEASE INDUCED BY CYTOTOXICS

Studies in human patients have been performed to investigate whether 5-HT is released during cytotoxic drug therapy. The urinary excretion of 5-HIAA (3) and, more recently, the plasma levels of CgA (4, 5), have been used as markers for serotonin release from EC cells. The gastrointestinal tract has the highest body content of serotonin and CgA, and the great majority is contained within EC cells. In EC cells, CgA is co-stored along with serotonin in the core of serotonin storage vesicles (6). Therefore, when a large amount of serotonin (and hence CgA) is released from the EC cells, this release is reflected by increases in plasma 5-HIAA and CgA, and in the urinary excretion of 5-HIAA (Figure 1). When serial samples are collected and processed for 5-HIAA and CgA, the time-course for the release of serotonin from EC cells can be estimated (3, 7, 8). These studies, in cancer patients, have demonstrated that strongly emetogenic cytotoxics release serotonin from EC cells, and that the time-course for the release of serotonin parallels that of emesis.

Measurements of the concentration of free serotonin in plasma have for the most part shown no significant changes (8, 9). In addition, platelet serotonin concentrations have been shown not to change after cytotoxic drug treatment (7, 8).

Therefore, all emphasis has been put on 5-HIAA and, more recently, on plasma CgA concentrations. These studies suggest that cytotoxics release serotonin from EC cells and not from platelets. We have proposed that free serotonin within the intestinal wall, is involved in triggering the emetic response. This explains why patients with carcinoid tumours (EC cell tumours) do not experience intense vomiting. Carcinoid tumours are not innervated, and consequently the anatomical and functional relationships between EC cells, vagal visceral afferent fibres, and the nucleus tractus solitarius and area postrema do not exist in the tumour. Thus, 5-HT released from malignant EC cells fails to activate vagal visceral afferent fibres.

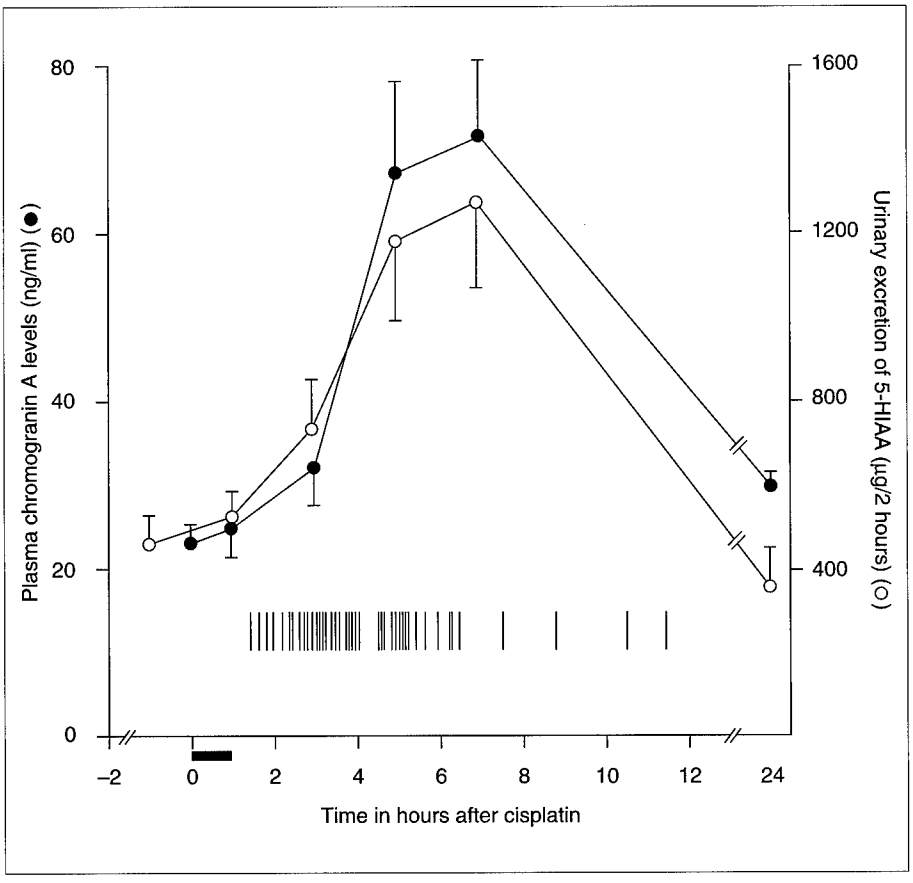

Figure 1. Pattern of emesis and changes in plasma CgA and in urinary 5-HIAA after high-dose cisplatin, in cancer patients. The amount of 5-HIAA was determined in urine collected during 2 hour periods, starting with the cisplatin infusion. Cisplatin was infused for over 1 hour starting at time zero. Vertical lines represent emetic episodes.

EFFECTS OF SEROTONIN-DEPLETING AGENTS

Another line of evidence in support of the role of serotonin in emesis induced by cytotoxics, comes from experiments where patients have been pretreated with a serotonin-synthesis inhibitor (Table 1) (10). The purpose of these experiments was to reduce the serotonin tissue concentrations, and then to observe the emetic response to cytotoxic therapy. PCPA, in doses of 6 g daily for 2 or 3 days before the administration of cisplatin, exerted a strong anti-emetic effect. Despite the use of high-dose cisplatin, patients treated with PCPA required no rescue anti-emetics, 4 out of 7 subjects did not vomit, 2 out of 7 experienced one episode of vomiting and one patient vomited twice, during the period when intense vomiting is normally expected (10–14 emetic episodes are usually observed if no anti-emetic is given). Together with this reduction in emesis, there was a 60–70% decrease in the daily urinary excretion of 5-HIAA and, more importantly, a marked reduction in cisplatin-induced increases in urinary 5-HIAA. Although based on a small number of patients, these results support the view that acute emesis after cisplatin-based therapy is serotonin-dependent, and that it seems to be induced by the release of serotonin from EC cells. Interestingly, in all animal species so far tested, PCPA preferentially depletes neuronal (CNS) serotonin stores. EC cells are more resistant to depletion after PCPA (1, 11). The observation that doses of PCPA which reduced urinary 5-HIAA by only 50% (a marker of GI serotonin synthesis and turnover), and failed to inhibit cisplatin-induced increases in urinary 5-HIAA, also failed to prevent cisplatin-induced emesis (10), is an argument in favour of the view that peripheral and not central serotonin participates in emesis associated with treatment with cisplatin.

CISPLATIN-INDUCED EMESIS

High-dose cisplatin induces nausea and vomiting in nearly all patients (3, 12). Two types of emetic responses have been described for high-dose cisplatin: acute and delayed emesis. Acute emesis is the early, intense period of emesis, which is

Table 1. Effects of PCPA on cisplatin-induced acute emesis.

	PCPA (n = 7)	Placebo (n = 14)
Complete response	57%	0%
Major response	43%	0%
Minor response	0%	36%
Failures	0%	64%

Cisplatin dose employed: 93 ± 3 mg/m^2 PCPA group; 78 ± 7 mg/m^2 placebo group. (PCPA data from (10) and placebo data from (3)).

prevented by prophylactic treatment with 5-HT_3 receptor antagonists (5-HT_3-dependent emesis) (3). This period of emesis is associated with increases in plasma and urine concentrations of 5-HIAA, and in plasma CgA concentrations (Figure 1) (3–5, 7, 8). In addition, acute emesis is blocked by pretreatment with PCPA (10). As discussed above, this evidence suggests that serotonin acting on 5-HT_3 receptors mediates the period of 5-HT_3-dependent emesis (acute emesis). The observation that cisplatin induces a large release of serotonin from EC cells, and that this release has a similar time-course to that of acute emesis, suggests that both events are highly related. Furthermore, with low doses of cisplatin (20–40 mg/m^2), both the amount of serotonin released and the intensity of emesis are less than observed with higher doses of the drug (8).

Cisplatin-induced delayed emesis, on the other hand, is sporadic, poorly responsive to 5-HT_3 receptor antagonists (5-HT_3-independent emesis), is not associated with increases in urinary 5-HIAA (7, 13), and appears more responsive to high-dose glucocorticoids. This type of emesis may be related to changes in gastrointestinal motility pattern, inner-ear toxicity, and/or mucosal damage induced by cisplatin.

NITROGEN MUSTARD-INDUCED EMESIS

In contrast to cisplatin, much less is known about the patterns and mechanisms of emesis associated with nitrogen mustard-based chemotherapy treatments. In a recent study we have shown that the most intense emesis is observed between 2 and 4 hours after the initiation of the nitrogen mustard infusion (4.9 ± 0.9 mg/m^2). This period of intense emesis (acute emesis) is associated with concomitant increases in the urinary excretion of 5-HIAA (5) and in the plasma concentration of CgA (5). The anti-emetic efficacy of 5-HT_3 receptor antagonists against nitrogen mustard has been demonstrated in clinical trials (1).

DACARBAZINE-INDUCED EMESIS

As with nitrogen mustard, little is known about the pattern and mechanisms of emesis associated with dacarbazine-based chemotherapy treatments. Our study has shown that the most intense emesis is observed between 2 and 5 hours after the initiation of the infusion of dacarbazine (193 ± 34 mg/m^2). This period of intense emesis is associated with concomitant increases in the urinary excretion of 5-HIAA (5, 8), and in the plasma concentration of CgA (5). Anti-emetic efficacy of 5-HT_3 receptor antagonists against dacarbazine-based regimens has been demonstrated in clinical trials (1). In summary, as for cisplatin and nitrogen mustard, the early period of intense emesis associated with dacarbazine treatment is 5-HT_3 receptor-dependent, and is associated with a large release of serotonin from EC cells. For these three agents, a similar mechanism of emesis is proposed.

CYCLOPHOSPHAMIDE- AND DOXORUBICIN-INDUCED EMESIS

The pattern of emesis occurring with cyclophosphamide treatment, in doses of about 500 mg/m^2, has been described (14). Cyclophosphamide is commonly administered in combination with other chemotherapeutic drugs. Cyclophosphamide, methotrexate and 5-fluorouracil (CMF), or cyclophosphamide, adriamycin (doxorubicin) and 5-fluorouracil (CAF), are some of the most frequently employed regimens. CAF induces an earlier onset and more intense emesis than CMF, and the anti-emetic efficacy of 5-HT$_3$ receptor antagonists is greater against CMF than CAF regimens (14). Therefore, doxorubicin, in doses greater than 40 mg/m^2, contributes significantly to the pattern and intensity of emesis observed with CAF regimens. The CMF regimens, therefore, better reflect the emetic response to cyclophosphamide.

Studies on serotonin metabolism revealed that cyclophosphamide-based chemotherapies do not induce changes in the urinary excretion of 5-HIAA, plasma CgA and platelet serotonin levels (5–8). These results suggest that, in contrast to cisplatin, dacarbazine and nitrogen mustard treatments, CAF and CMF regimens do not induce important increases in serotonin release from EC cells. It should be noted that neither 5-fluorouracil, nor methotrexate (drugs commonly used in combination with cyclophosphamide), increase the release of serotonin from EC cells, as judged by the lack of increase in the urinary excretion of 5-HIAA and in the plasma concentration of CgA.

Like high-dose cisplatin, a component of the emesis associated with cyclophosphamide-based chemotherapies, particularly CAF regimes, does not respond well to 5-HT$_3$ receptor antagonists. We have demonstrated that, even after repeated, high doses of ondansetron, a significant proportion of patients receiving CMF and CAF regimens experienced five or more emetic episodes, and/or required rescue anti-emetic therapy because of the severity of their emesis (14). Furthermore, complete response rates (i.e. no emesis) after high-dose ondansetron were 72% for CMF and 59% for CAF treatments. In addition, 7% and 16% of placebo-treated patients failed to vomit after CMF and CAF regimens (14). We suggest that, like high-dose cisplatin, emesis associated with cyclophosphamide–doxorubicin regimens has two components: 5-HT$_3$ receptor-dependent and 5-HT$_3$ receptor-independent. Interestingly, the period of 5-HT$_3$ receptor-dependent emesis observed with CMF and CAF regimens is not accompanied by measurable increases in serotonin release from EC cells (see below).

5-HT$_{1A}$ RECEPTORS AND CYTOTOXIC-INDUCED EMESIS

The anti-emetic efficacy of buspirone, a partial agonist at 5-HT$_{1A}$ receptors, has been recently evaluated (Table 2). Buspirone is the only 5-HT$_{1A}$ receptor agonist available for clinical use. At a dose of 120 mg (2 × 60 mg doses, 30 minutes apart), which is several times greater than that required for anxiolytic action, buspirone failed to protect against cisplatin-induced emesis. Despite this negative result, experiments

Table 2. Comparative anti-emetic effects of oral ondansetron and oral buspirone in cisplatin-induced emesis in cancer patients.

	Buspirone (n = 10)	Ondansetron (n = 9)	Placebo (n = 14)
Complete response	0%	56%	0%
Major response	10%	33%	0%
Minor response	30%	11%	36%
Failures	60%	0%	64%

Emesis was evaluated for the first 24 hours after cisplatin.

Cisplatin doses (mg/m^2): 85 ± 4 *versus* buspirone; 81 ± 6 *versus* ondansetron; and 78 ± 7 *versus* placebo.

Buspirone (120 mg p.o.) and ondansetron (16 mg p.o.). (Placebo data from (3)).

with full agonists (when available) must be conducted to provide a definitive answer on the participation of 5-HT_{1A} receptors in cytotoxic-induced emesis.

CONCLUSIONS

Evidence from clinical studies suggests that cisplatin-, nitrogen-mustard-, and dacarbazine-based regimens induce a large, measurable release of serotonin from EC cells. The release of serotonin occurs with a time-course that is similar to that of acute emesis (5-HT_3 receptor-dependent emesis; emesis blocked by 5-HT_3 receptor antagonists), suggesting that serotonin released from EC cells triggers emesis by acting on 5-HT_3 receptors, probably located on visceral afferent fibres. In CAF and CMF regimens, 5-HT_3 receptor-dependent emesis develops without a measurable (large), concomitant release of serotonin from EC cells. These results clearly indicate the existence of different mechanisms for emesis induced by cytotoxics. It is possible that emesis induced by cyclophosphamide and doxorubicin is more dependent on central 5-HT_3 receptors. The mechanisms by which cisplatin and cyclophosphamide regimes induce emesis insensitive to 5-HT_3 receptor antagonists (5-HT_3 receptor-independent emesis, or delayed emesis) are unknown, and are currently under active investigation. The recent observation that selective neurokinin-1 (NK_1) receptor antagonists prevent 5-HT_3 receptor-dependent and independent forms of emesis in animal models, suggests that substance P acts as a final, common mediator of emesis (see Chapter 25). Clinical studies on the anti-emetic properties of NK_1 receptor antagonists are forthcoming.

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Chapter 15

What is the involvement of 5-HT_3 receptors in postoperative nausea and vomiting?

L. Strunin

INTRODUCTION

General anaesthetics were first administered in 1846. Within months it became clear that such anaesthetics could be followed by postoperative nausea and vomiting (PONV). This was ascribed to the drugs themselves and was thought to be caused by their sympathetic stimulating effects; rates of PONV of 70–80% were often described (1). In 1956 the volatile anaesthetic halothane was introduced into clinical practice, and there was a dramatic fall in the incidence of PONV, to 25–30% (1). The increasing use of potent opioids (such as fentanyl) during both general and regional anaesthesia/analgesia increased the incidence once more (1–3). PONV is usually taken to mean following a general anaesthetic. However, it is worth noting that PONV occurs in relation to local and regional anaesthesia/analgesia as well, although to a lesser extent. The incidence increases when mixture of drugs is used (3).

The current move to the rapid turnaround of inpatients, and the expansion of day-care has focused on PONV as a cause for the inability of patients to be discharged. An additional factor is that general anaesthesia is now so safe that there is increasing interest in anaesthetic morbidity as opposed to mortality (4). It is also true that the development of potent 5-HT_3 receptor blocking drugs, with their remarkable effect on nausea and vomiting associated with cytotoxic drugs, has prompted interest in their possible efficacy in PONV.

INCIDENCE OF PONV

The incidence of PONV is very variable. PONV is generally self-limiting and is rarely life-threatening; nevertheless it can be the most distressing part of what should be an event-free general anaesthetic and operation (5). In a study of 16,000 patients given four different modern anaesthetics in a random fashion (6), a prospective follow-up gave an overall incidence of PONV of 18%; this rose to 25% in those patients given fentanyl, and severe emesis occurred in 0.12%. In a retrospective study of 29,000 patients, the overall incidence was 25% and was highest in the age group of 6–10 years (7).

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AETIOLOGY OF PONV

It seems clear that PONV is multifactorial, and combinations of the following seem relevant: volatile and gaseous anaesthetics, opioids, hypoxia, pain, hypotension, vestibular reflexes, female sex, age, type of surgery, psychological factors, previous history of PONV or motion sickness, and stimulation of the oropharynx (2, 3, 8). Which of these are more important is difficult to predict in an individual patient. However, it has been shown (9) that certain fixed patient factors are more significant than others (i.e. . gender, history of previous PONV, postoperative opioids and a history of motion sickness). Logistic regression analysis in a study of some 500 patients given a standardized general anaesthetic showed the following (9):

Logit postoperative sickness
= 5.03 + 2.24 (postoperative opioids)
+ 3.97 (previous sickness history)
+ 2.4 (gender) + 0.78 (motion sickness)
+ 3.2 (gender × previous sickness history)

As a working example, consider a male patient with a history of PONV and motion sickness who also receives postoperative opioids:

Logit postoperative sickness
= 5.03 + 2.24 (1) + 3.97 (1) + 2.4 (0) + 0.78 (1) – 3.2 (0 × 10)
= $1.96/e^{1.96}$
= 7.09

Probability of sickness in 24 hours
= 7.09/8.09
= 0.876 or 87.6% (95% confidence limits)

The example above describes a patient at very high risk of PONV, who would require an extremely efficacious drug to prevent PONV. A small number of such patients not randomly assigned in clinical trials of anti-emetic drugs will make a big difference to the results, especially if small numbers are compared and the drugs are not very effective. Therefore, it is essential that these fixed patient factors are taken into account as risk stratification in assigning patients to study groups in trials of anti-emetic drugs, otherwise it may not be possible to determine their anti-emetic effects with any accuracy.

MODELS OF PONV

There is no animal model of human PONV. Anti-emetic drug studies have therefore concentrated on human models (e.g. females undergoing gynaecological surgery, children having strabismus surgery) with a known high incidence of PONV. However, there has rarely been risk stratification of the other patient factors outlined above. Recently a subunit of the 5-HT_3 receptor was cloned, and expression of the cDNA encoding this protein in *Xenopus* oocytes resulted in homomeric ion channels. In this model, 5-HT_3 receptor function was enhanced by low concentrations of halothane and isoflurane, but not propofol (10). As this accords with the clinical observation that propofol (an intravenous anaesthetic) appears in some studies to reduce the incidence of PONV in day care (11), the *Xenopus* oocyte model may be useful in the assessment of anti-emetics in the future. It is perhaps of interest that propofol has been used to try to control the nausea and vomiting associated with antineoplastic chemotherapy (12), but in this regard it is worth noting that propofol is a potent general anaesthetic even at low doses, and may have, in patients who are unwell, profound deleterious effects on the cardiorespiratory system.

5-HT_3 RECEPTOR ANTAGONISTS AND PONV

"The precise mechanism through which 5-HT and 5-HT_3 receptors contribute to the control of PONV is unknown, but their involvement is demonstrated by the anti-emetic effect of ondansetron" (11). Before the advent of ondansetron, anti-emetics that acted at dopamine (prochlorperazine, droperidol), muscarinic cholinergic (scopolamine, promethazine) and histamine (cyclizine, diphenhydramine, promethazine) receptors, or drugs with gastrointestinal prokinetic activity (metoclopramide, domperidone, cisapride; some of which have weak 5-HT_3 antagonistic action) were most popular for PONV. None is fully effective and all have significant side-effects. Current studies are directed at establishing the place of ondansetron in the management of PONV.

A MedLine search (Lopez and Mathieu, *Anesthesiology* 1994, ASA Abstracts) between 1980 and 1993 revealed 69 articles detailing randomized trials comparing ondansetron with placebo for treatment of PONV in the first 24 hours following surgery. However, after applying criteria to make the articles acceptable for meta-analysis, only six articles plus two abstracts were deemed suitable. A total of 1739 patients were described, and the ondansetron-treated patients were 3.0 and 2.5 times more likely to be free from postoperative vomiting and postoperative nausea, respectively, than the placebo-treated patients. In addition, the ondansetron-treated patients were 4.5 times less likely to require rescue medication than the placebo-treated patients. In these studies, ondansetron was given prophylactically either as premedication or during the general anaesthetic. In a study in 30 centres in the USA

(Clayborne, *Anaesthesia* 1994; **49** (Suppl): 24–29) 1000 patients were studied after they had developed nausea or vomiting in the first 2 hours after general anaesthesia in the post-anaesthetic recovery unit. The patients were randomized to receive either intravenous ondansetron 1, 4 or 8 mg, or placebo and then followed for 24 hours. The dose of 4 mg ondansetron seemed to be optimal, but was only effective (statistically better than placebo, but not clinically very impressive) in female patients. This fits with the concept of the high risk male patient described above who would need a very effective drug, and clearly ondansetron as used in this study does not have an effect.

THE FUTURE

As PONV is multifactorial, it is hardly surprising that ondansetron is not as effective in this situation as it is against nausea and vomiting induced by chemotherapy. In the latter, there is good evidence that 5-HT_3 receptor antagonists are very effective in prevention. The gold standard anti-emetic in PONV has been droperidol (2), but even at modest dosage the drug has significant side-effects. There have been no good comparative trials of droperidol and ondansetron to date, particularly none with risk stratification. These are needed. Although ondansetron is more expensive than droperidol, it has fewer side-effects, particularly on single dosing (4 mg), which seems most effective in adults. There are a number of other 5-HT_3 receptor antagonists and these require study to see if they are better than ondansetron. Because of the multifactorial nature of PONV, it is likely that mixtures of drugs blocking various receptors may be better than the single drug approach.

The question posed in this paper was what is the role of 5-HT_3 receptor blocking drugs in PONV? At present one cannot answer this question – indeed, as the mechanisms of PONV unfold, it may be that 5-HT_3 receptors have only a minor role in the problem. However, the advent of specific blocking drugs has precipitated an immense interest in PONV and focused attention on its management and eventual prevention. This has been of great benefit to patients who in the past were told "it's the anaesthetic" and received neither sympathy nor treatment.

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Chapter 16

Preclinical differences in 5-HT_3 receptor antagonist characteristics

G. J. Sanger

INTRODUCTION

Within a single species, only one 5-HT_3 receptor is currently recognized, although differences are acknowledged to occur between species. This conclusion cannot be challenged until the structure and conformations of the ligand-gated ion channel are better understood. However, certain inconsistencies in the functional efficacies of 5-HT_3 receptor antagonists, within a species, have led to the introduction of the term '5-HT_3-like receptor' to accommodate the possibility that a subtype of the receptor might exist. This chapter summarizes some of these inconsistensies that have been derived from preclinical studies and seeks to explain them in terms of additional pharmacological activities, pharmacokinetic peculiarities, or the existence of a putative 5-HT_3-like receptor/ion channel complex. (To evaluate the potential significance of these findings in terms of clinical anti-emetic efficacy, refer to Chapter 17.)

5-HT_3 RECEPTOR ANTAGONISTS WITH ADDITIONAL PHARMACOLOGICAL PROPERTIES

Insofar as they have been tested, most 5-HT_3 receptor antagonists are highly selective in their action. Well-known exceptions include metoclopramide and renzapride, which also promote gastrointestinal motility via activation of the 5-HT_4 receptor. This additional activity reduces their ability to inhibit cytotoxic-evoked emesis (see below). By contrast, tropisetron antagonizes at the 5-HT_4 receptor, but only at high, clinically irrelevant doses. Compounds that antagonize in low concentrations at both 5-HT_3 and 5-HT_4 receptors are now available (e.g. FK-1052; 1), but the consequences of combining these two properties are not yet clear.

Azasetron (Y-25130) can enhance the amplitude of electrically evoked, cholinergically mediated contractions of guinea-pig isolated ileum, in a concentration-dependent manner (10^{-7}–10^{-4} M) (2). Although this effect is qualitatively similar to 5-HT_4 receptor activation, the activity of azasetron cannot be prevented by the 5-HT_4 receptor antagonist, SB 204070. Nevertheless, by analogy with compounds that do activate the 5-HT_4 receptor as well as antagonize at the 5-HT_3 receptor, this excitatory effect of azasetron could explain the observed

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reduction in its anti-emetic efficacy in ferrets, relative to granisetron (2). Interestingly, this type of excitatory effect has also been observed in rat stomach with ondansetron (pEC_{50} 6.0) (3), a compound that also has no meaningful affinity for the 5-HT_4 receptor.

ATYPICAL ANTI-EMETIC PROPERTIES OF 5-HT_3 RECEPTOR ANTAGONISTS

Differences in the anti-emetic profiles of different 5-HT_3 receptor antagonists have already been well documented ((2, 4); see also Chapters 5 and 19). In terms of pre-clinical data, the most striking difference was reported from one study, in which either linear or non-linear anti-emetic dose–response curves were obtained for different 5-HT_3 receptor antagonists in ferrets (4), in addition to differences in potency and duration of action. The possible explanations for some of these differences are explored in this section.

Additional pharmacological activity

In our original experiments with renzapride and MDL 72222, we noticed that the anti-emetic efficacy of renzapride was less than for MDL 72222, in spite of their equivalent efficacies as 5-HT_3 receptor antagonists and an approximately 10-fold advantage for renzapride in terms of potency. Accordingly, the authors suggest (5) that the ability of renzapride to increase gastrointestinal motility and/or activate the 5-HT_4 receptor, reduced the anti-emetic efficacy of 5-HT_3 receptor antagonism. This argument has again been used to explain the unexpected lower efficacy of azasetron in ferrets, compared with granisetron, as studies *in vitro* suggest that azasetron may also promote gastrointestinal motility (2).

At present, the mechanism by which gut-motility stimulants may reduce anti-emetic efficacy is unknown. However, one possibility is presented by the discovery that the 5-HT_4 receptor is also located on the vagus nerve in rats and mice. Activation of this receptor may be involved in at least some forms of emesis (6). Consequently, it does not seem unreasonable to suppose that compounds that activate this receptor will at least partly oppose the anti-emetic effects of 5-HT_3 receptor antagonism.

Receptor-kinetic differences

In the rat vagus nerve, granisetron, tropisetron and BRL 46470 antagonize the 5-HT-evoked depolarization in an insurmountable manner. By contrast, the antagonism by ondansetron is surmountable. These differences may arise because of a difference in the rates at which the compounds dissociate from the receptor (7). The slower the dissociation, the more unfavourable the competition with 5-HT for the receptor, a factor that may contribute to an increase in the duration of emetic activity *in vivo* (8). Further studies that compare the rates of dissociation of different 5-HT_3 receptor

antagonists from the human 5-HT_3 receptor (see Chapter 9) are now required, so that any possible differences can be related to clinical anti-emetic studies.

Pharmacodynamic differences

In anaesthetized ferrets, Bingham and King (9) measured the influence of granisetron and ondansetron on the vagally mediated gastric corpo-antral reflex, in which inflation of the gastric corpus results in an increase in the amplitude of antral contractions. Low doses of granisetron (0.1, 0.5, and 2.0 mg/kg i.v.) reduced both the reflex and the baseline antral contractility. However, the same doses of ondansetron increased the contractility of the antrum. These findings seem remarkably similar to those of Buchheit *et al.* (3), who found that ondansetron (pEC_{50} 6.0), but not tropisetron or granisetron, increased electrically evoked, cholinergically mediated contractions of rat isolated stomach.

Bingham and King (9) discussed their findings in terms of the potential involvement of vagally mediated gastric reflexes in the aetiology of nausea. This possibility is consistent with the suggestion that the anti-emetic efficacy of 5-HT_3 receptor antagonists (against cytotoxic therapy) is reduced if these compounds also promote gastrointestinal motility.

5-HT_3-LIKE RECEPTORS IN THE INTESTINE?

5-HT_3 receptor antagonists have little or no effect on normal upper-gut function, but the fact that these agents can cause constipation suggests that the 5-HT_3 receptor is involved in the physiology of human lower-bowel function and possibly in defaecation. In this lower-bowel activity, differences in the efficacies of different compounds are emerging that cannot easily be explained by their pharmacodynamic or kinetic properties. Accordingly, the term '5-HT_3-like receptor' has been introduced to embrace the potential existence of a receptor subtype.

Among the most powerful suggestions for the existence of a 5-HT_3-like receptor was that obtained in the experiments of Sanger and Wardle (10) in guinea-pig tissues. After demonstrating the efficacy of granisetron, tropisetron and BRL 46470 against the 5-HT-induced, cholinergically mediated contraction of the isolated colon (respective pA_2 values: 8.5, 8.5, 7.9), it was found that low concentrations of granisetron in the bathing solution surrounding an isolated colon slowed or stopped its ability to expel endogenous faecal pellets in a spontaneous manner. Tropisetron was also effective, albeit at higher concentrations than granisetron; BRL 46470 had no effect. These observations were consistent with the abilities of the compounds to reduce the number of faecal pellets expelled from fed, conscious guinea-pigs (10). They were also consistent with similar studies in fed rats (YM060 and granisetron reduced faecal expulsion or 5-HT-evoked diarrhoea, whereas ondansetron was less effective; 11), and in human volunteers (12). In the latter study, orocaecal and whole-gut transit time were unaffected by BRL 46470, and a low incidence of

constipation was reported, in marked contrast to the ability of other 5-HT_3 receptor antagonists to cause constipation in the same experimental paradigm.

Differences between the efficacy of 5-HT_3 receptor antagonists have also been reported in models of intestinal fluid secretion and absorption, an area of gastrointestinal function that is strongly influenced by 5-HT acting on 5-HT_3 and other 5-HT receptors (13). Close intra-arterial injections of granisetron or ondansetron reduce the volume of fluid secreted into the jejunum of anaesthetized rats after intraluminal administration of the heat-stable enterotoxin of *Escherichia coli*, whereas tropisetron is without effect (14). Similarly, granisetron, but not tropisetron, reduces diarrhoea in sennoside-treated rats (15).

Finally, in the vascularly perfused, tetrodotoxin-treated guinea-pig isolated ileum, low concentrations of granisetron, tropisetron or MDL 72222 decrease the release of 5-HT from the mucosal enterochromaffin cells, whereas ondansetron is without effect (16). These studies are again strongly indicative of the existence of a 5-HT_3-like receptor, similar in characteristics to that described by the intestinal secretion experiments, above.

5-HT_3-LIKE RECEPTORS AND VISCERAL PAIN?

The identification of MDL 72222 was driven by the proposal that high doses of metoclopramide reduced the pain of migraine by an antagonist action at the 5-HT_3 receptor on the vascular afferent neurones that transmitted the sensation of pain. In pilot trials, MDL 72222 was found to be efficacious when administered during the headache phase of a migraine attack (17). However, the concept has subsequently been thrown into doubt and confusion by studies that demonstrate a lack of anti-migraine efficacy for tropisetron (18) and zatosetron (19), but a suggestion of activity for granisetron in pilot studies (20).

Pilot studies with granisetron also suggested that this compound could reduce rectal hypersensitivity in patients with the irritable bowel syndrome (IBS). These studies were undertaken as a blinded trial by recording the sensations evoked by rectal distension (21). However, as for the trials in migraine patients, the use of other drugs failed to replicate these observations. Most notable was the failure of ondansetron to affect visceral perception evoked by rectal distension in IBS patients (22) or consistently to reduce the pain recorded by ambulatory IBS patients (23). Indeed, in the latter studies, some patients reported an exacerbation of pain following ondansetron treatment.

The possibility that 5-HT_3 receptor antagonists might control visceral pain has been studied in various rat models, and compounds have been administered either systemically or into the spinal cord. In our studies we focused on measuring the potential analgesic properties of systemically administered antagonists in anaesthetized rats subjected to noxious duodenal (24) or colo-rectal distension (25). Under these conditions, 'pain' is estimated by recording the associated

pseudoaffective reflexes, in this case a systemic vascular depressor response evoked by the noxious stimulus. In both tests, the efficacy of ondansetron was found to be substantially less than that recorded for granisetron, an observation that was particularly surprising in view of the ability of both of these compounds to suppress completely the 5-HT-evoked von Bezold-Jarisch reflex and show CNS activity in the same species (5). These studies were recently extended by measuring the sensitivity of conscious rats to a balloon distension of the colo-rectum (26). In these studies, the effects of distension were observed as abdominal muscle contraction, with the threshold occurring around 10–40 mmHg distension. We found that granisetron and ondansetron did not affect the visceromotor thresholds to colorectal distension in normal rats. However, when the reflex was sensitized by prior injection of a low dose of the 5-HT precursor, 5-hydroxytryptophan, analgesic activity could be observed for granisetron but marked differences in the efficacy of the different antagonists became apparent. Thus, granisetron, zatosetron, bemesetron and renzapride were approximately equi-effective analgesic agents. Metoclopramide was also effective, an observation consistent with the many clinical reports of visceral analgesic properties for this compound (26). By contrast, the analgesic properties of tropisetron occurred only at relatively high doses, whereas ondansetron and BRL 46470 were without effect. These differences in the efficacies of 5-HT_3 receptor antagonists forced us to introduce the term '5-HT_3-like receptor' because of the difficulty of explaining our observations in terms of pharmacodynamic or kinetic properties.

Our observations of visceral pain in the rat models are generally consistent with the clinical observations obtained with many of the compounds in patients suffering from two different forms of visceral pain, migraine or IBS. The exception is zatosetron, which is effective in conscious rats but not in migraine patients (19).

STRUCTURAL VARIATIONS IN THE 5-HT_3 RECEPTOR/ION CHANNEL COMPLEX

To rationalize the 5-HT_3-like receptor, it is essential to compare functional data with structural and/or conformational variations in the 5-HT_3 receptor/ion channel complex. Sufficient variations in the latter are now being accumulated, which suggest that this will eventually be achieved (see Chapter 9).

Binding sites

In NG108-15 cells, modification of tryptophan residues with N-bromosuccinimide reduces [^{3}H]-zacopride binding in a manner that can be protected by pre-incubation with various 5-HT_3 receptor agonists and antagonists, but not with *m*-chlorophenylbiguanide or with zacopride itself. These results suggest the existence of different recognition sites on the 5-HT_3 receptor/ion channel complex (27). Similarly, Steward *et al.* (28) report that, compared with granisetron and

ondansetron, BRL 46470 labels additional specific binding sites in human embryonic kidney cells stably transfected with the murine 5-HT_3 R-A_s receptor subunit, and in rat cortex/hippocampus preparations. By contrast, BRL 46470 fails to label the granisetron-sensitive binding sites in human putamen.

Characterization of the affinity of various compounds for the 5-HT_3 receptor binding sites in mouse brain (cerebral cortex) and gut (ileum) suggest that more than one recognition site may occur within a single species (29). Differences in affinity were particularly apparent for RS-42358-197, (R)-zacopride, 1*m*-chlorophenylbiguanide, (R)-YM060 and MDL 72222.

Channel conductance

5-HT_3 receptor-gated currents recorded in mammalian autonomic and central (hippocampus) neurones, exhibit unitary conductances in the range of 8–20 pS. Usually, only one conductance value is obtained in each tissue, but in guinea-pig enteric (submucosal) neurones two distinct channels of 10 and 17 pS have been observed (30). The co-existence of two structurally distinct forms of the 5-HT_3 receptor in the rat hippocampus has also been indirectly implied (but not demonstrated) by Jones and Surprenant (30).

A low-conductance (0.1–0.6 pS) 5-HT_3 receptor has consistently been found in mouse neuroblastoma cells, contrasting with the larger conductances recorded for the receptor in other mouse tissues (31). At present, this difference seems peculiar to the mouse 5-HT_3 receptor (but see below). However, as phosphorylation of the channel in mouse neuroblastoma cells can affect the conductance (32), it becomes a possibility that different states of the 5-HT_3 receptor channel might exist in all species, according to the level of intracellular protein-kinase activity induced by 5-HT or by other neurotransmitters acting at different receptor sites.

Structure

Two splice variants of the mouse 5-HT_3 receptor have been characterized, termed 5-HT_3R-A and 5-HT_3R-A_s. The R-A_s form has six amino acids deleted from the putative large intracellular loop between the predicted third and fourth transmembrane regions; to date, the shorter variant has not been found in the human genomic library. This structural variation seems to have no effect on the potency or efficacy of 5-HT_3 receptor agonists and antagonists, with the exception of a reduced maximal current evoked by the 5-HT_3 receptor agonist 2-methyl-5-HT in the expressed 5-HT_3R-A_s form (31). If this observation can be related to other reports of partial activation of the 5-HT_3 receptor by 2-methyl-5-HT (rat vagus nerve, 33; mouse neuroblastoma NIE-115 cell line, 34; guinea-pig superior cervical ganglia, 35; mouse superior cervical ganglia, 34), it would suggest that both splice variants of the 5-HT_3 receptor may exist in a range of different species. A recent and successful search for the splice variants by PCR in rat brain and nodose ganglion now supports this suggestion (36). Interestingly, the authors found that although the ratio of the two forms was reasonably consistent between the adult rat tissues, the

proportion of the longer form was dependent on the time of embryonic development. Further work is now required, particularly in terms of investigating the patho-pharmacological significance of the 5-HT_3 receptor channel splice variants in adult and embryonic tissues, and in looking for a similar existence in human tissues.

SUMMARY AND CONCLUSIONS

Only one 5-HT_3 receptor is formally recognized, but sufficient, inexplicable differences in 5-HT_3 receptor antagonist pharmacology are emerging to justify the use of the term '5-HT_3-like receptor'. These differences are especially significant when obtained *in vitro* during conditions that minimize potential variations in pharmacokinetic properties (guinea-pig colon, 10; guinea-pig enterochromaffin cells, 16). However, important differences have also been obtained *in vivo* under conditions where compounds have been administered by close intra-arterial injection (enterotoxin-induced fluid secretion in rat jejunum, 14), or in species in which 'classical' 5-HT_3 receptor pharmacology has been obtained in one system (rat von Bezold-Jarisch reflex) but not in another (rat visceral pain experiments; 24–26).

None of the individual differences described above constitutes proof of the existence of a 5-HT_3 receptor subtype, as these must first be matched with structural differences in the receptor/ion channel complex. However, when described together, the need to search for structural and conformational differences in the 5-HT_3 receptor/channel complex becomes persuasive. The future could, therefore, reveal the existence of a 5-HT_3 receptor subtype, a pharmacologically significant interpretation of the splice variant structures, a better understanding of the control of channel conductance via other cellular influences on channel phosphorylation, or a combination of all three. The challenge will be to correlate these findings with pathophysiological differences in 5-HT_3 receptor function and with the efficacies of different 5-HT_3 receptor antagonists.

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Chapter 17

Are there any true clinical differences between 5-HT_3 receptor antagonists?

M. S. Aapro

INTRODUCTION

The initial description in 1986 by Miner and Sanger (1) of the anti-emetic efficacy of 5-hydroxytryptamine M-receptor (later called the 5-HT_3 receptor) antagonists, motivated several companies to develop such agents for the control of cancer treatment-related emesis (see Chapter 4). The first of these agents, ondansetron, progressed to licensing and widespread clinical use within 5 years, a remarkable achievement in drug development. This was largely a reflection of the efficacy and safety of these new drugs in the prevention and control of cancer therapy-related emesis, which became evident during the first clinical studies and allowed a very rapid progression through the different stages necessary for approval by registration authorities (2). Thus, we have in 1995 three agents available to adult and paediatric oncologists and radiotherapists for prevention of cancer therapy-related emesis: granisetron, ondansetron and tropisetron. Several other 5-HT_3 receptor antagonists are under development, or have already received a licence in a single country. These compounds represent, alone or in combination with corticosteroids, the best choice for the prevention of acute emesis in adult cancer patients undergoing moderate to highly emetogenic chemotherapy, in paediatric oncology, and in radiation therapy-induced emesis. The purpose of this paper is to compare and review the clinical activity of the three agents mentioned above in the setting of acute emesis, that is, within the first 24 hours after chemotherapy.

PRECLINICAL EVIDENCE OF DIFFERENCES BETWEEN THE 'SETRONS': A CLINICIAN'S PERSPECTIVE

Potency in animal and *in vitro* models

The relative potency of the 5-HT_3 receptor antagonists has been studied in physiological models, and in animals challenged with chemotherapeutic agents or ionizing radiation. Several publications have shown that there are differences in the potency of granisetron, ondansetron and tropisetron in a number of physiological models, and the results are summarized in Table 1. They show that granisetron and

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Table 1. Potency and affinity of 5-HT_3 receptor antagonists.

Model	Bezold-Jarisch ED_{50}	Rabbit vagus depolarization pA_2	Guinea-pig ileum pA_2
Granisetron	0.7	10.86	8.1
Ondansetron	2–3.6	9.4	7.3
Tropisetron	0.4–3.0	10.2	7.9–8.3

Modified from (3) and (4).

tropisetron are relatively similar in potency, whereas ondansetron is a somewhat less potent agent (3, 4).

For the clinician, this observation is interesting but, in view of the excellent safety profile of these drugs, not particularly relevant for if one agent is less potent than another, we should just use more of it to obtain the same result.

Receptor-binding

The receptor-binding profile of all three agents shows a high degree of selectivity for the 5-HT_3 receptor, with only minor affinities for other receptors: granisetron has detectable affinity at the 5-HT_{1A} receptor, ondansetron has similarly minimal affinity for 5-HT_{1B} and 5-HT_{2C} receptors, and adrenergic α_1 and μ opioid receptors, and tropisetron is a weak antagonist at 5-HT_4 receptors. However, from a clinical viewpoint none of these appears to be of significance (5).

Anti-emetic potency, duration of action and dose-responsiveness

Granisetron, ondansetron and tropisetron have all been evaluated extensively in ferrets given cisplatin, or exposed to ionizing radiation. The data (5, 6) from these studies confirm that there are differences in potency between ondansetron, granisetron and tropisetron, and in addition to this, granisetron has the longest duration of action (Table 2). There are also some data to suggest that the dose–response curve for ondansetron in animal models is non-linear (6). Finally, in rat isolated vagus nerve preparations, the depolarization elicited by 5-HT (largely a 5-HT_3 receptor-mediated event) is inhibited by ondansetron in a surmountable

Table 2. Preclinical therapeutic models: comparison of anti-emetics.

	Potency	Dose–response	Duration
Granisetron	5–10	Linear	2×
Ondansetron	1	?Non-linear	1
Tropisetron	3–5	Linear	?

Adapted from (6).

manner (i.e. it is sufficient to increase the serotonin dose to overcome the antagonism), whereas tropisetron and granisetron are insurmountable antagonists ((7, 8); see also Chapter 9).

However, despite all these differences that can be identified in animal models (see also Chapter 16), there is no evidence that this makes any difference in clinical usage, other than influencing the choice of drug dosage.

CLINICAL SIMILARITIES AND DIFFERENCES

Pharmacokinetics and metabolism

In agreement with the above observations of a longer duration of anti-emetic action of granisetron in animal models, the terminal half-life of granisetron in human subjects is 9–11 hours, compared with 4.5 hours for ondansetron and 7.3 hours for tropisetron (2, 4). Tropisetron, however, is metabolized by the hepatic cytochrome P-450 2D6 isoenzyme, a well-characterized oxidative pathway that is subject to genetic polymorphism, and in about 7% of Caucasians the activity of the enzyme is deficient. It is in these so called 'poor metabolizers' that the plasma half-life of tropisetron is around 33 hours. This does not, however, modify the clinically recommended dosage in this group of patients (4). Recent studies show that ondansetron metabolism may also involve this same pathway, but to a much smaller extent (9) and it is not of clinical significance.

Side-effect profile

The profiles of side-effects caused by the three 5-HT_3 receptor antagonists are very similar. Tabona, (for granisetron; (10)), Bryson, (for ondansetron; (11)), and de Bruijn, (for tropisetron; (12)), report that minor headaches (which respond to paracetamol) are observed in 10–15% of patients, and constipation in less than 10% of the patients. Other side-effects attributable to the 5-HT_3 receptor antagonists are very unusual (as opposed to those from concomitant cytotoxic therapy, e.g. diarrhoea). The only way of looking for differences in the safety profile of the three drugs is to make a compilation of the data presented in comparator randomized studies. Table 3 shows the side-effect profiles as they have been reported in such studies (13–21). It is striking to observe how some of the side-effects are reported at extremely different frequencies from one study to another. This obviously reflects the study methodology, and the definition of the given side-effect in the particular study. In some studies, nurses were asked to interview the patients using a preset questionnaire, in other studies investigators asked more general questions, and in others, patients were expected to report side-effects spontaneously. Finally, not all side-effects were taken into account, as discussed by Jantunen *et al.* (14). There is no statistical difference between the agents in the reported side-effects, which in any case were always of a minor nature. The only possible exception to this is the incidence of headache in the report by Martoni *et al.* (18).

Table 3. Side-effect profile of 5-HT_3 receptor antagonists in comparator studies.

Author	Agent	Headache	Constipation	Any
G.E.A.R.	Ond 8 mg	12%	1%	15%
	Ond 32 mg	10%	0%	15%
	Gran 3 mg	7%	2%	9%
Jantunen	Ond 8 mg	35%	NA	NA
	Gran 3 mg	35%	NA	NA
	Trop 5 mg	34%	NA	NA
Bonneterre	Ond 8 mg i.v. + Ond 2 × 8 mg p.o.	27%	52%	NA
	Gran 3 mg	34%	41%	NA
Noble	Ond 8 mg i.v. + Ond 2 × 8 mg p.o.	17%	16%	63%
	Gran 3 mg	14%	15%	61%
Mantovani	Ond 24 mg i.v.	< 20%	NA	NA
	Gran 3 mg	< 20%	NA	NA
	Trop 5 mg	< 20%	NA	NA
Martoni	Ond 3 × 8 mg i.v.	26%	NA	NA
	Gran 3 mg	5%	NA	NA
Gebbia	Ond 16–24 mg i.v.	9%	17%	NA
	Gran 3 mg	4%	7%	NA
Marty	Ond 32 mg i.v.	21%	10%	82%
	Trop 5 mg	18%	10%	72%
Campora	Ond 8 mg i.v. + Ond 2 × 4 mg p.o.	0%	NA	5%
	Trop 5 mg	5%	NA	NA

Ond, ondansetron; Gran, granisetron; Trop, tropisetron; G.E.A.R., Group d'Etude des antagonistes des récepteurs 5-HT_3.

Efficacy

Nine studies that compare the efficacy of these agents in the prevention of acute chemotherapy-induced nausea and vomiting have been reported in some detail (13–21). Several other studies are ongoing or are unavailable for review. Table 4 summarizes the results of the published trials. Only one of them has looked at patients undergoing moderate to highly emetogenic cisplatin-based single-day chemotherapy (13). This study compared intravenous administration of either 3 mg of granisetron, 8 mg of ondansetron, or 32 mg of ondansetron, in 496 patients. It confirms previous European observations about the lack of difference between the two doses of ondansetron (21). A subgroup analysis of the previously published European study suggests that patients treated with high doses of cisplatin (> 100 mg/m^2) may not do quite as well with the 8 mg as with the 32 mg dose of ondansetron, although this difference does not reach statistical significance. It is, however, in agreement with the American group (22) who showed the superiority of 32 mg of ondansetron in highly emetogenic chemotherapies.

Table 4. Efficacy of 5-HT_3 receptor antagonists in comparator studies.

Author	Agent	Chemotherapy	Control	Preference
G.E.A.R.	Ond 8 mg	CDDP	59%	Parallel
	Ond 32 mg	> 49 mg/m^2	51%	
	Gran 3 mg		56%	
Jantunen	Ond 8 mg	'Moderate'	69%	17%
	Gran 3 mg		80%	42%
	Trop 5 mg		75%	15%
Bonneterre	Ond 8 mg i.v. + Ond 2 × 8 mg p.o.	'Moderate'	45%	34%
	Gran 3 mg		52%	39%
Noble	Ond 8 mg i.v. + Ond 2 × 8 mg p.o.	'Moderate'	40%	26%
.	Gran 3 mg		44%	34%
Mantovani	Ond 24 mg i.v.	CDDP	65%	No data
	Gran 3 mg	> 80 mg/m^2	72%	
	Trop 5 mg		44%	
Martoni	Ond 3 × 8 mg i.v.	CDDP	50%	22%
	Gran 3 mg	> 50 mg/m^2	59%	49%
Gebbia	Ond 16 mg i.v.	'moderate'	69%	Parallel
	Gran 3 mg		67%	
Gebbia	Ond 24 mg i.v.	CDDP	52%	Parallel
	Gran 3 mg	> 70 mg/m^2	49%	
Marty	Ond 32 mg i.v.	CDDP	65% (N:62%)	
		> 50 mg/m^2		Parallel
	Trop 5 mg		54% (N:66%)	
Campora	Ond 8 mg i.v. + 2 × 4 mg p.o.	FAC/FEC	60%	Parallel
	Trop 5 mg		50%	

Ond, ondansetron; Gran, granisetron; Trop, tropisetron; N, nausea control (the other percentage refers to vomiting only); FEC/FAC: 5-fluorouracil, (epi)adriamycin, cyclophosphamide; G.E.A.R, Group d'Etude des antagonistes des récepteurs 5-HT_3; CDDP, *cis*-diamminedichloroplatinum. See text for details and critique.

The study published by a Finnish group (14), includes, like the G.E.A.R. study, patients who might have had previous chemotherapies, and was conducted in 161 non-cisplatin-treated patients using an open design. This open design, plus the lack of data on the profile of the patients in each arm concerning their previous anti-emetic experience before entering the study, makes it difficult to accept the striking differences that were reported as being related to a difference in efficacy. The study presented by Bonneterre on behalf of the French Northern Oncology Group (15) was, like the Finnish study, of an open, cross-over design. It compared patients undergoing moderately emetogenic chemotherapy and reported that the control of nausea and vomiting was similar over 24 hours of observation between the two arms of the study. A more methodologically rigorous comparator study is that of Noble *et al.* (16). This gives the data from a Granisetron Study Group trial

that was a double-blind, double-dummy study in 309 patients who were naive to chemotherapy. Reporting results for the 5-day observation period of patients undergoing 5 days of moderately emetogenic chemotherapy with cisplatin (20 mg/m^2) or ifosfamide, this study demonstrated no difference between a single administration of granisetron and a multiple-dose administration of ondansetron, a schedule chosen according to the then applicable data sheet for ondansetron (as was the case for the study presented by Bonneterre). Two interesting studies were reported at the American Society for Clinical Oncology meeting in 1994. Mantovani *et al.* (17) conducted an open study in 86 chemotherapy-naive patients treated for head and neck cancer. Patients received high-dose cisplatin-based chemotherapy and were naive to chemotherapy when randomized to receive one of three regimens; a single intravenous administration of 24 mg ondansetron, 3 mg of granisetron, or 5 mg of tropisetron. As in the Jantunen report, tropisetron was apparently somewhat less effective than the other two agents. However, this paper is open to the same criticism as the Finnish report: it was an open study, and some prognostic factors like alcohol consumption are not mentioned, although patients were well matched for age, sex and other criteria, according to the authors. Martoni *et al.* (18) have looked at 82 chemotherapy-naive patients who received several types of moderately emetogenic cisplatin-containing drug regimens. Their patients were randomized between a single dose of granisetron on day 1, or 3 × 8 mg ondansetron i.v. on day 1, followed on the second day by 2 × 8 mg p.o. This study shows no difference between groups in the complete control of vomiting. Gebbia (19) has conducted a large open single-institution trial in two groups of chemotherapy-naive patients. The first group of 164 patients were scheduled to receive moderately emetogenic chemotherapy and were treated with ondansetron, 16 mg i.v., or granisetron, 3 mg i.v., and the other group of 182 patients (due to receive moderately high doses of cisplatin) were treated with ondansetron, 24 mg i.v., or granisetron, 3 mg i.v. Again in this study there was no difference between the two agents. Marty *et al.* (20) conducted the only other double-blind, double-dummy study reported to date. They compared a single infusion of 32 mg of ondansetron with 5 mg i.v. of tropisetron in 231 chemotherapy-naive patients. The patients, treated with cisplatin-containing regimens, had somewhat better protection from emesis with ondansetron compared with tropisetron, but had similar protection against nausea. Unfortunately, although this seems to have been included in the study design, the authors do not report the control achieved against nausea and vomiting together. The figures given in Table 4 for 'control' usually represent the control of vomiting only, which is a less satisfactory end-point than control of both nausea and vomiting. Finally, in a small study comprising 40 women with breast cancer undergoing 'FAC' or 'FEC' chemotherapy (5-fluorouracil, (epi)adriamycin, and cyclophosphamide), who were not all chemotherapy naive, Campora (21) has shown no statistically significant difference between ondansetron and tropisetron, in a 'worst-day' analysis.

PATIENT PREFERENCE

As discussed by several authors, the advantage of crossover studies is that patient preference can be evaluated. This is acceptable only if the studies are blind, as this subjective evaluation can be influenced by many factors. Nevertheless, Table 4 includes the results of the above mentioned studies that looked at patient preference. In the unblinded Bonneterre study (15), patient preference was marginally in favour of granisetron (but not statistically significant), possibly because it was a single injection instead of an injection and tablets, as for ondansetron. The double-blind, double-dummy study by Noble *et al.* (16) avoided this type of bias, and this study concluded that patients preferred granisetron, when a preference was expressed, but it should be noted that the majority expressed no preference between granisetron and ondansetron (Table 4). Although all studies in which this comparison was possible show a trend in favour of granisetron, the criticisms expressed above about most studies do not allow any final conclusion to be drawn.

CONCLUSIONS

There is no doubt that the three 5-HT_3 receptor antagonists currently in widespread clinical use are highly effective anti-emetic agents. The preclinical differences between granisetron, tropisetron and ondansetron are real, but whether these differences are clinically significant is a matter of debate. The studies discussed above do not represent the final answer. We await the results of properly designed double-blind, double-dummy studies in highly emetogenic therapies to reach a conclusion; and the large 1000-patient trial conducted in Italy by Roila *et al.*, due to be presented in late 1995, should go a long way towards providing an answer. This study compares 8 mg of ondansetron with 3 mg of granisetron, both in combination with dexamethasone. As previous studies have shown the superior efficacy of such a combination, it seems likely that if there are any minor differences between the 5-HT_3 receptor antagonist drugs, they will disappear when they are used in combination with dexamethasone. Cost–benefit analyses (24, 25) are in favour of the use of these drugs as first-line agents in emetic chemotherapy for the control of acute emesis.

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Chapter 18

Limitations in the efficacy of 5-HT_3 receptor antagonists

D. G. Warr

INTRODUCTION

The 5-HT_3 receptor antagonists have brought about a substantial advance in supportive care, because of superior efficacy and adverse effect profile compared with alternative anti-emetics. Nonetheless, controlled trials have shown that these compounds fail to prevent vomiting during the first 24 hours in approximately 50% of patients receiving high-dose cisplatin (1–6). The category of moderately emetogenic chemotherapy is much more heterogeneous, but most studies show that approximately 70% of patients receiving this chemotherapy will have an early onset of vomiting despite the use of ondansetron or granisetron (1, 7–12).

Although these complete protection rate figures show a substantial improvement upon results achieved with other agents, it should be kept in mind that they are a conservative estimate of the true problem. Approximately one in four patients will start to vomit beyond the first 24 hours (3, 7, 11, 12) – a problem that may not be influenced by continued administration of 5-HT_3 receptor antagonists. Still other patients may not begin to vomit until the second or later cycle of chemotherapy. There is conflicting evidence about whether this latter problem is a common occurrence. A study by Kaizer *et al.* in patients receiving moderately emetogenic chemotherapy suggests that the frequency of this phenomenon may depend upon the definition of control (12). If one includes as responders those patients who have one or two episodes of emesis following the first cycle of chemotherapy, then by cycle three the number of responders decreases by 15–25%. If one only includes those with no emesis after the first cycle, then the drop-off in response rate is only 4–10%. According to these data, patients with complete control of emesis following the first cycle continue to do well with subsequent chemotherapy. Those patients who experience even one to two episodes of emesis, however, have a substantially greater risk of experiencing at least three episodes of emesis with subsequent cytotoxic therapy.

Although the 5-HT_3 receptor antagonists as single agents are imperfect, there are clinical data demonstrating that these results can be improved. There are also results emerging from the animal models that suggest that the next few years may bring us much closer to the goal of complete prevention of chemotherapy-induced emesis.

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OPTIMIZING 5-HT$_3$ RECEPTOR ANTAGONIST ACTIVITY

A logical first step in trying to improve the efficacy of the 5-HT$_3$ receptor antagonists is to explore dose–response relationships. The work of Soukop *et al.* (13) and Smith *et al.* (8) with granisetron showed that four-fold increases in granisetron dosage did not improve the number of patients free from emesis. The studies with ondansetron provide conflicting results. In patients receiving high-dose cisplatin, Seynaeve *et al.* (6) and Ruff *et al.* (5) could find no evidence that efficacy was altered over a four-fold dose range. In a study of similar design, however, Beck *et al.* demonstrated superiority of a single 32 mg dose of ondansetron over an 8 mg dose (14). The explanation for this discrepancy is unclear and at this time it is not known if a single 32 mg dose of ondansetron is superior to an 8 mg dose.

A second issue to which some attention has been devoted is the schedule of drug administration. Ondansetron has a median terminal half-life of less than 4 hours and might, therefore, be more effective when given in repeated doses. Comparisons of a single dose of ondansetron with either a bolus dose followed by a continuous infusion or with multiple doses, have not, however, demonstrated any advantage with the more complicated regimens (6, 14). Indeed the study by Beck *et al.* suggested that a single dose of 32 mg was more effective than a dose of 0.15 mg/kg for three doses (14). This is an interesting finding, because the latter schedule will deliver a total of approximately 30 mg for a 70 kg patient. Thus, a single dose of ondansetron in the first 24 hours is as effective as more complicated approaches. It has been assumed that 5-HT$_3$ receptor antagonists with longer terminal half-lives than ondansetron would not demonstrate improved efficacy with repeat dosing over the first 24 hours.

Despite the efficacy of 5-HT$_3$ receptor antagonists in the first 24 hours following chemotherapy, there has been some uncertainty about their value for preventing emesis that is delayed in onset. Although a number of studies have attempted to draw conclusions about the utility of administering these compounds beyond day one, most suffer from the methodological pitfall that the comparator groups differ in the anti-emetic therapy that was administered in the first 24 hours. The influence of the initial anti-emetic therapy on delayed-onset emesis has been demonstrated in a study by Roila, in which the anti-emetic therapies compared (ondansetron + dexamethasone *versus* metoclopramide + dexamethasone + diphenhydramine) differed only in the first 24 hours (15). Despite the fact that both groups received the same doses of dexamethasone and metoclopramide beyond 24 hours, the results on day 2 were significantly better for those patients who had received ondansetron and dexamethasone as anti-emetic therapy on the first day (no emesis in 83.8% *versus* 69.1%). Therefore, the only studies that should be used to draw conclusions about the value of using a medication beyond day one to prevent delayed-onset emesis are those in which identical anti-emetic therapy has been administered to both groups in the first 24 hours.

There are three studies with a design that is appropriate for evaluating the benefits of administering 5-HT$_3$ receptor antagonists beyond the first day of

chemotherapy – two with ondansetron and one with granisetron. In a small study by Gandara *et al.* in patients receiving high-dose cisplatin, there was no significant difference in the proportion of patients free of emesis on ondansetron versus metoclopramide, although there was a slight trend in favour of ondansetron (16). Kaizer *et al.* reported the results of an NCI Canada Clinical Trials Group trial in which patients were randomized to receive ondansetron or placebo for days 2–5 (12). All patients received chemotherapy other than high-dose cisplatin and the anti-emetics were delivered in a double-blind fashion. The group that received ondansetron beyond day 1 had a significantly higher complete response rate than did the group that received placebo. This Canadian group has recently completed a study in patients receiving high-dose cisplatin, and found that granisetron, 1 mg twice daily by mouth on days 2–7 did not add to the efficacy of dexamethasone, 8 mg by mouth twice daily, for the prevention of delayed onset emesis (Personal communication) At this point it is difficult to reconcile the apparently contradictory results quoted above, but the explanation may rest with patient selection and whether or not dexamethasone has been given for several days following chemotherapy. At best, administration of 5-HT_3 receptor antagonists beyond the first 24 hours appears to be of modest utility. To improve our ability to control emesis, we must look beyond the issues of dose, frequency and duration of administration of these agents.

AGENTS THAT ADD TO 5-HT_3 RECEPTOR ANTAGONIST EFFICACY

There is substantial evidence that we can improve control of emesis by adding agents to the 5-HT_3 receptor antagonists. The efficacy of these interventions is categorized in this chapter as *established* (two or more double-blind randomized trials), *possible* (one or more controlled trials), or *potential* (evidence from animal models only) (see Table 1).

The strongest evidence that an intervention has an additive anti-emetic effect is with the use of dexamethasone. Several studies demonstrate that the addition of this steroid can increase the complete response rate at 24 hours in patients receiving high-dose cisplatin by 20–30% (3, 4, 17). A recent study by Carmichael *et al.* demonstrated similar benefits in patients receiving moderately emetogenic chemotherapy (18). Therefore, there is no doubt that glucocorticoids enhance the efficacy of 5-HT_3 receptor antagonists in the prevention of early-onset emesis.

As mentioned above, delayed-onset emesis is a problem for patients treated with 5-HT_3 receptor antagonists, just as it has been for patients receiving high-dose metoclopramide (19). There are no data to indicate whether the occurrence of this troublesome phenomenon after these new anti-emetics can be prevented by the prolonged administration of glucocorticoids. The strikingly positive result in a study by Kris *et al.*, with continued oral dexamethasone following high-dose metoclopramide (19), has led many clinicians to prescribe steroids for several days

Table 1. Interventions that improve 5-HT_3 receptor antagonist efficacy.

Established benefit	Possible benefit	Potential benefit (animal model only)
Dexamethasone Dopamine receptor antagonist	TENS at the P6 acupuncture point Proprofol infusion	Non-NMDA receptor antagonists Fentanyl NK_1 receptor antagonists

following high-dose cisplatin chemotherapy, irrespective of the anti-emetic administered on day 1. Oncologists should now consider glucocorticoids to be a standard accompaniment to 5-HT_3 receptor antagonists. In patients receiving high-dose cisplatin there is a reasonable basis for prescribing several days of dexamethasone in an attempt to prevent delayed-onset emesis.

Another intervention for which there is evidence of additive benefit from randomized trials is the use of the dopamine receptor antagonist metopimazine. Herrstedt and colleagues have carried out studies in patients receiving moderately emetogenic chemotherapy (primarily cyclophosphamide, methotrexate and 5-fluorouracil (CMF); 20) as well as cisplatin-containing chemotherapy (21), and concluded that metopimazine significantly improves the efficacy of ondansetron both in the first 24 hours and the subsequent 5–6 days. As modest doses of dopamine D_2 receptor antagonists are both inexpensive and well tolerated, the demonstration of an effect that is additive to that of 5-HT_3 receptor antagonists is of great interest.

There are two interventions for which there is less evidence of efficacy, but which nonetheless merit further study – propofol infusions and transcutaneous electrical nerve stimulation (TENS). Subhypnotic doses of the anaesthetic agent propofol were reported to have outstanding anti-emetic activity in a study by Borgeat (22). This study enrolled 20 patients who had experienced more than five episodes of emesis, despite the use of dexamethasone plus either ondansetron or granisetron in previous cycles of high-dose cisplatin chemotherapy. In 85% of patients given propofol, there was no emesis in the first 24 hours. In the 24–72 hour period post-chemotherapy, 75% of patients remained free of emesis. Only one patient experienced sedation. Although this was not a randomized trial, these results are impressive for prevention of both acute and delayed vomiting, because the patient population was refractory to the best available standard therapy. A limiting factor in its application may be the feasibility of administering propofol as a continuing infusion in outpatients. Further studies are needed, not only to confirm the efficacy and safety of this intervention but also to determine the mechanism of action of propofol.

Another intervention with an unknown mechanism of action is the use of TENS at the P6 acupuncture point. McMillan reported the results of an unblinded crossover

study in 16 patients who received 5-day cycles of chemotherapy with low-dose cisplatin (23). All patients received ondansetron with two consecutive cycles of chemotherapy, and during one of the two cycles TENS was also administered. No patients experienced more emesis on the cycle in which the TENS was administered. Nine patients had one to two episodes of vomiting with ondansetron alone and eight of these patients had no emesis when TENS was added. When expressed in terms of complete response rates, the control rate was 69% with the combined treatment versus 19% with ondansetron alone. Although TENS technology is widely available, this intervention has not been tested by other groups or adopted in practice. The nature of the intervention makes it difficult to carry out blinded studies but further evaluation of this non-toxic treatment is desirable.

NOVEL APPROACHES IN THE ANIMAL MODEL

It was in the ferret that the potential value of 5-HT_3 receptor antagonists for chemotherapy-induced emesis was first demonstrated. In this same animal model, anti-emetic efficacy has now been demonstrated for approaches that are not dependent upon serotonin. Three such interventions will be briefly reviewed – non-NMDA receptor antagonists, opioid receptor agonist therapy and neurokinin$_1$ (NK_1) receptor antagonist therapy. As both opioid and NK_1 receptor agonists are covered by other authors, these topics will be mentioned only briefly.

The excitatory amino acid receptors have recently been demonstrated to have effects on cisplatin-induced emesis in ferrets (24, 25). The subgroup of these receptors known as non-NMDA receptor antagonists has prevously attracted some interest in the management of experimental wind-up pain caused by nerve injury. In a study of their anti-emetic potential by Fink-Jensen, the non-NMDA receptor antagonists, 6-cyano-7 nitroquinoxaline-2, 3-dione (NBQX) and 6-nitro-7-sulphamobenzo(f)quinoxaline-2, 3-dione (CNQX), were able to prevent vomiting in 5/6 and 3/5 ferrets, respectively, during the 4 hour observation period (25). When these compounds were co-administered with a dose of ondansetron that was only partially effective as a single agent, all ferrets were protected from vomiting. These results were achieved with no observable adverse effects. Although this new class of compounds is being primarily explored in animals for the treatment of neuropathic pain and brain ischaemia, they merit further investigation for their possible anti-emetic effects.

A class of anti-emetic drugs that has been around for a much longer period of time than the non-NMDA receptor antagonists is the opioids. Although opioids will induce nausea in some patients, for several decades it has been recognized that predosing animals with one opioid may inhibit emesis due to another agent. Barnes *et al.* have recently demonstrated that, in the ferret, fentanyl in a dose of 10–80 μg/kg can completely inhibit not only emesis caused by apomorphine or copper sulphate but also that which is caused by cisplatin (26). The explanation offered is that μ opioid

receptor agonism in the brainstem by this lipophilic agent results in supression of emesis that does not appear to be dependent upon the nature of the emetic stimulus.

The applicability of these observations on opioids as anti-emetics to humans is far from clear. There has been a small study suggesting that the μ opioid receptor antagonist naloxone can increase emesis in patients receiving chemotherapy (27), and it is possible that an anti-emetic effect of TENS is opioid-based, as analgesia mediated by low frequency TENS may be antagonized by naloxone. Consistent with an anti-emetic role for opioids is a small study by Lissoni *et al.* suggesting that an enkephalin analogue (which would bind to μ and ∂ receptors) decreased emesis caused by cisplatin (28). Although these observations are of interest, the effect of opioids on respiratory and cognitive function and their tendency to cause emesis in some patients will make it difficult to evaluate opioids as anti-emetics.

Other compounds that may prove useful in the future are antagonists of a class of receptors that have substance P as a ligand. Tattersall *et al.* demonstrated striking anti-emetic results in the ferret model of cisplatin-induced emesis using an intravenous injection of the NK_1 receptor antagonist CP-99,994 (29). There was a dose-dependent supression of emesis, with the only reported toxicity at the highest dose being very transient hypotension. In contrast to the opioid results, this novel class of compounds is attracting considerable interest.

SUMMARY

The heterogeneity of anti-emetic response to the 5-HT_3 receptor antagonists indicates that there must be 5-HT_3 receptor-independent mechanisms. There is evidence that the anti-emetic effect of 5-HT_3 receptor antagonists is improved by adding dexamethasone or metopimazine and possibly through the use of TENS at the P6 acupuncture site or propofol infusions. Observations with cisplatin-induced emesis in animal models suggest that a variety of non-serotonin-based therapies may further improve our ability to decrease the still troublesome problem of chemotherapy-induced nausea and vomiting.

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Chapter 19

Does 5-HT play a role in the delayed phase of cisplatin-induced emesis?

L. Grélot, S. Milano and H. Le Stunff

INTRODUCTION

Nausea and vomiting (i.e. emesis) are the side-effects most feared by cancer patients receiving chemotherapy (1). In humans, cytotoxic drugs such as cisplatin cause severe and often prolonged bouts of vomiting (2). Vomiting occurring within approximately the first day following the administration of antineoplastic agents corresponds to the acute phase of vomiting (3). The pathophysiology of this phase has been extensively studied during the past decade (4, 5) and this type of emesis can now be relatively well controlled with 5-HT_3 receptor antagonists (e.g. granisetron, ondansetron or tropisetron). The less intense but more prolonged bouts of vomiting beginning after the first day and lasting up to 1 week, correspond to the delayed phase of vomiting (3) and this has proved more resistant to inhibition by anti-emetic drugs (6–8). The neural and pharmacological mechanisms responsible for the development of delayed vomiting remain obscure.

In this chapter we will first describe a suitable animal model in which to study the pathogenesis of delayed emesis, then we will review the effects of 5-HT_3 receptor antagonists and 5-HT_4 receptor agonists on both acute and delayed cisplatin-induced emesis. Finally, the temporal changes in the gastrointestinal levels of serotonin induced by cisplatin treatment will be presented. Some results from this series of experiments suggest that serotonin might play a role in eliciting vomiting during the delayed phase of cisplatin-induced emesis.

CISPLATIN-INDUCED EMESIS IN THE PIGLET

Fourteen weaned piglets (39–70 days old) of either sex, weighing up to 12 kg, were surgically implanted with a cannula in the jugular vein. After 4–5 days' recovery, piglets were hydrated (100 ml/hour over 10 hours) with sterile isotonic saline plus furosemide (0.1 mg/kg), and then given cisplatin (5.5 mg/kg i.v., i.e. approximately 100–150 mg/m^2). Piglets were observed continuously for the following 60 hours during which animals received continuous intravenous infusions of glucose and polyionic solutions. In some animals, blood samples taken at various times revealed that cisplatin induces myelosuppression, which takes the form of leucopenia, thrombocytopenia and anaemia. In addition, animals allowed to recover from the

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experiments exhibited a survival time of at least 3 weeks before they were sacrificed. This indicates the absence of acute lethal toxicity of cisplatin in the piglet under our experimental conditions.

All animals treated with a high dose of cisplatin experienced both acute and delayed emesis (Figure 1A). In the acute phase of emesis, the first vomiting occurred with a latency of 2.15 ± 0.69 hours (range 0.74 to 10.78) after cisplatin administration. Emetic intensity reached a peak (5 vomits/hour) within 2 hours and then decreased rapidly, so that no vomiting episodes were observed between 16 and 18 hours (Figure 1B). The mean number of vomiting episodes during the first 16 hours was 18.1 ± 1.9. Delayed emesis started at around 18 hours and was observed to last until 58 hours (Figure 1B). Emetic intensity of the delayed phase reached a peak (1.2 vomits/hour) between 21 and 22 hours, and the mean number of vomiting episodes during the whole of the delayed phase was 9.1 ± 2.3.

EFFECT OF 5-HT$_3$ RECEPTOR ANTAGONISTS ON CISPLATIN-INDUCED EMESIS

All piglets (n = 48) included in this experimental series were naive to both cytotoxic and anti-emetic drugs. All drugs were dissolved in 20 ml of sterile isotonic saline and administered over 15 minutes. The data are expressed as the means ± standard error. The statistical significance of differences in the latency of emetic events was assessed with the Mann-Whitney U-test, comparing control and treated animals. An analysis of variance followed by Bonferroni's test was performed for the other parameters evaluated. Significance was accepted at $p < 0.05$.

Single initial administration

Twenty-six piglets received a single intravenous injection of either granisetron (0.25 mg/kg, n = 3; 0.5 mg/kg, n = 3; 2 mg/kg, n = 3; or 7 mg/kg, n = 7), or ondansetron (0.5 mg/kg, n = 3; 2 mg/kg, n = 3; and 7 mg/kg, n = 4) 15 minutes before cisplatin.

All piglets, except two, responded to cisplatin with both acute and delayed emesis. Pretreatment with granisetron or ondansetron provided a benefit against acute emesis. Granisetron and ondansetron significantly increased the latency to the first emetic event in a dose-dependent manner. At a dose of 7 mg/kg the latency to the first emetic event was 12.48 ± 0.96 hours (range 10.03 to 18.53) and 10.67 ± 1.72 hours (range 7.35 to 16.46), for granisetron (Figure 2A) and ondansetron, respectively. The severity of the acute phase was reduced significantly only with granisetron at a dose of 7 mg/kg. The severity of the delayed phase remained unchanged with granisetron, irrespective of the dose, but paradoxically increased significantly with the high dose (i.e. 7 mg/kg) of ondansetron. The origin of this discrepancy between the anti-emetic profiles of the two drugs remains obscure. However, as the cumulative severity (i.e. acute + delayed severities) during 60 hours in animals receiving single initial doses of a 5-HT$_3$ receptor antagonist was statistically similar to that of control piglets (i.e. those

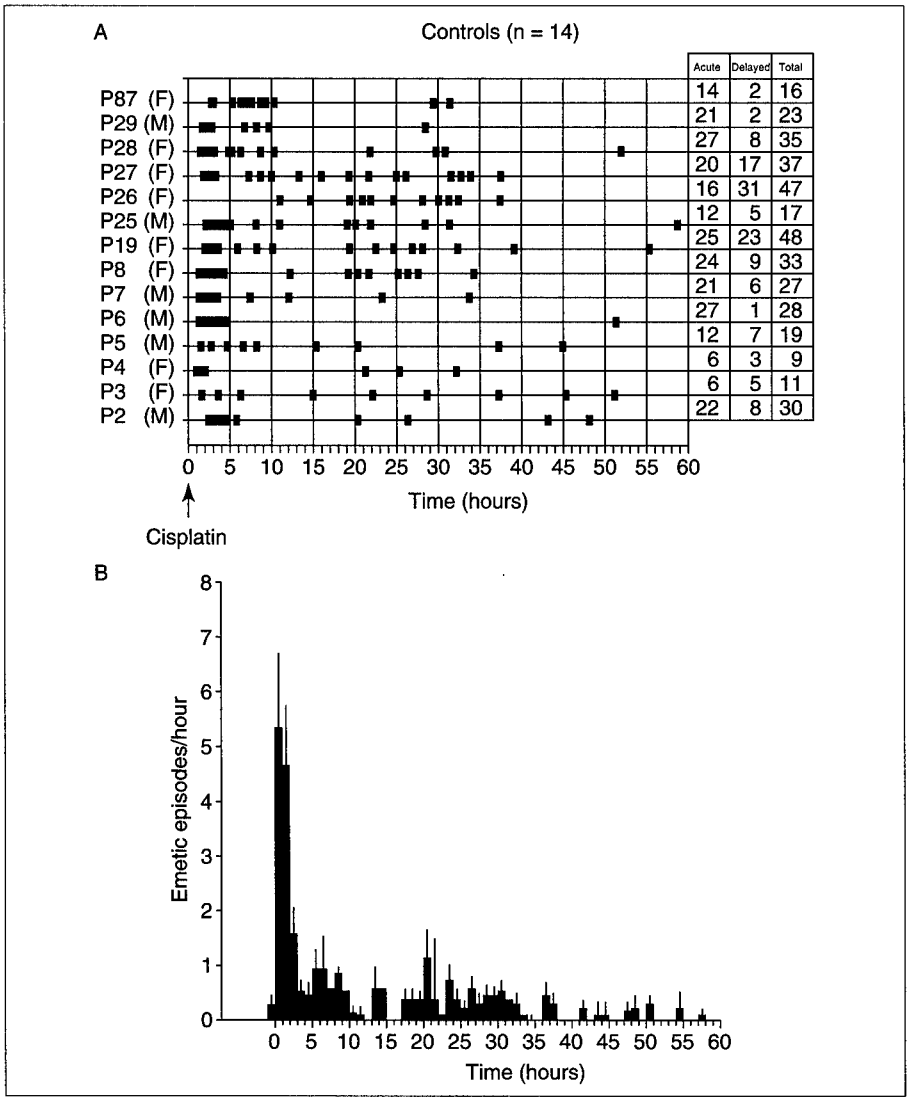

Figure 1. A: Pattern during the 60-hour observation period of the cisplatin-induced emetic episodes in control piglets (i.e. not treated with anti-emetic). Due to the scale, each black rectangle represents either a single emetic episode or a series of emetic episodes. Totals of vomiting episodes occuring during the acute (i.e. T0–T16), the delayed (i.e. T18–T60) and the total chemotherapy course (i.e. cumulative, T0–T60) are given for each animal in the three columns on the right. (M, male; F, female).

B: Distribution of the emetic episodes exhibited during the 60-hour observation period by control piglets (n = 14). Each column represents the mean ± SEM for a 1-hour interval. In each panel, cisplatin (5.5 mg/kg, i.v.) was administered at T = 0 (arrow).

not treated with an anti-emetic), one could speculate that some emetic events normally seen in the acute phase are merely delayed by treatment with the 5-HT_3 receptor antagonists.

Seven other piglets received 40 mg of dexamethasone during the chemotherapy course, in addition to the initial single high dose (7 mg/kg) of granisetron (Figure 2B). Dexamethasone was administered (20 mg) just before granisetron, and then 12 hours (10 mg) and 36 hours (10 mg) following cisplatin injection. In this population, the mean latency of the first emetic event increased significantly to 14.73 ± 2.01 hours (range 8.42 to 21.45). In contrast, the cumulative severity (16.3 ± 4.3 emetic episodes) remained statistically similar to that of control piglets.

Repetitive administration

Granisetron and ondansetron, administered as single initial doses, alone or in combination with dexamethasone, appeared inadequate in reducing the cumulative severity during the 60 hours of the observation period. This might be because of their short half-lives in the piglet. The dose–response curves for the two 5-HT_3 receptor antagonists revealed that single initial administrations of 1 mg/kg and 3.5 mg/kg of granisetron and ondansetron, respectively, would provide a mean protection against emesis for approximately 5 hours. Hence, in eight piglets, we administered in addition to an initial dose (1 mg/kg) of granisetron, a supplementary injection (same dose as the first one) every 5 hours during 30 hours (i.e. cumulative dose 7 mg/kg; Figure 2C). Similarly, seven other piglets received repetitive injections of 3.5 mg/kg of ondansetron (i.e. cumulative dose 24.5 mg/kg, Figure 2D). Statistical evaluations were performed as described above.

The mean latency of the first emetic event under repetitive administration of granisetron (44.17 ± 6 hour; range 15.18 to 60) was significantly longer than in control animals (i.e. not treated with anti-emetic). Moreover, repetitive administration of granisetron was beneficial in significantly reducing the severity of both the acute and delayed phases of vomiting. Consequently, the cumulative severity decreased significantly to 9.3 ± 4 emetic episodes. In three out of eight piglets, the multiple dosing regimen produced complete control of emesis (i.e. no vomiting throughout the chemotherapy course).

Repetitive injections of 3.5 mg/kg of ondansetron appeared less effective. Compared with control piglets, the mean latency of the first emetic event increased significantly to 20.34 ± 5.12 hours (range 4.33 to 40.18). However, the cumulative severity (29.3 ± 4.4 emetic episodes, range 15 to 42) remained unchanged. In addition, this treatment did not provide complete control in any animals.

EFFECT OF A 5-HT_4 RECEPTOR AGONIST ON CISPLATIN-INDUCED EMESIS

All piglets (n = 19) included in this experimental series were naive to both cytotoxic and anti-emetic drugs. All drugs were dissolved in 20 ml of sterile isotonic saline

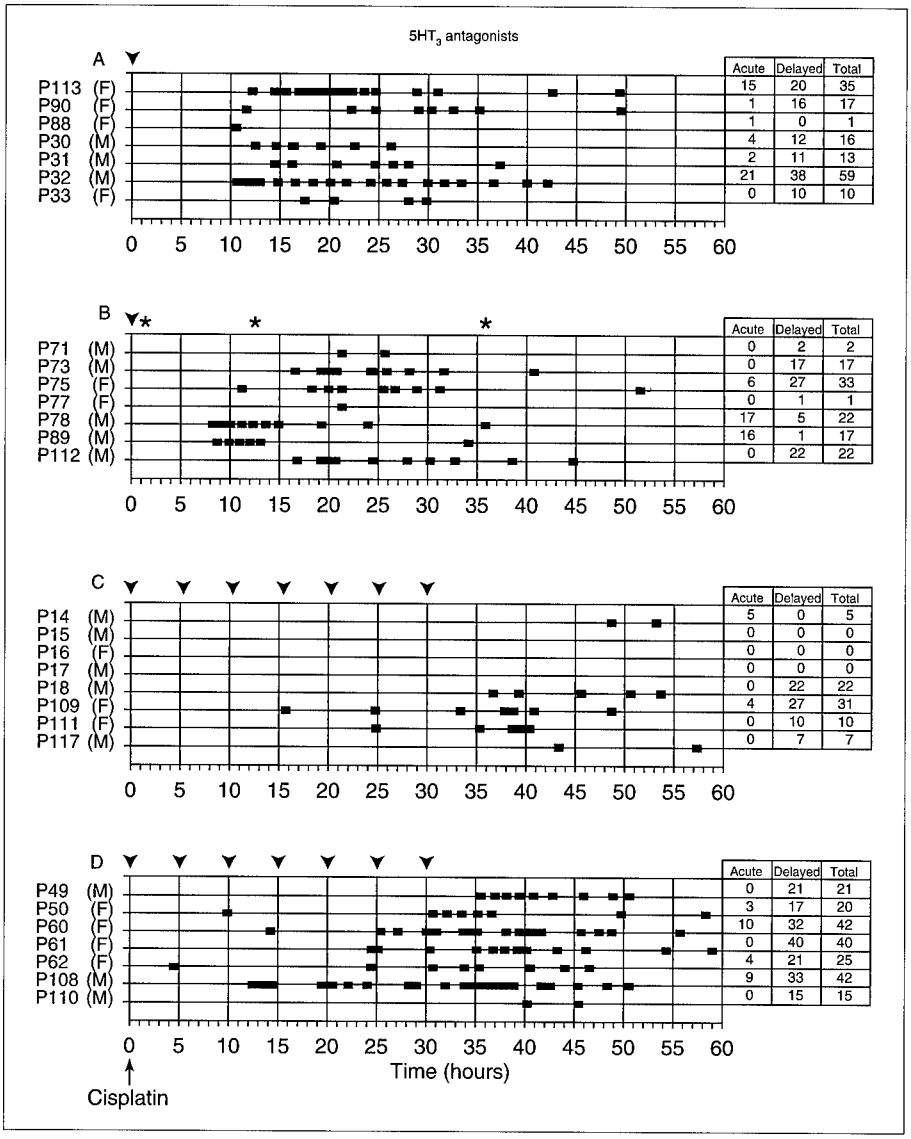

Figure 2. Pattern during the 60-hour observation period of the emetic episodes in piglets treated with a 5-HT_3 receptor antagonists (i.e. 'setrons').

A: Animals receiving a single initial dose (7 mg/kg) of granisetron.

B: Animals receiving a single initial dose (7 mg/kg) of granisetron plus dexamethasone (20 mg at T0 and 10 mg at T12 and T36).

C: Animals treated repeatedly (seven injections) with granisetron (1 mg/kg).

D: Animals treated repeatedly (seven injections) with ondansetron (3.5 mg/kg). Same representation as in Figure 1A. Arrowheads and asterisks above panels indicate the timing of injections of 'setrons' and dexamethasone, respectively.

and administered over 15 minutes. Statistical evaluations were performed as described above.

Single initial administration

Twelve piglets received a single intravenous injection of the 5-HT_4 agonist, BIMU 1 (2 mg/kg, n = 6, and 4 mg/kg, n = 6) 15 minutes before cisplatin.

All piglets treated with a single dose of 2 mg/kg of BIMU 1 (Figure 3A), responded to cisplatin with both acute and delayed emesis. The mean latency of the first emetic event increased significantly to 8.02 ± 1.3 hours (range 5.33 to 14.02). In contrast, the cumulative severity (31 ± 6 emetic episodes, range 19 to 50) remained unchanged. With a single dose of 4 mg/kg, all animals exhibited a delayed phase of emesis but one did not vomit during the acute phase (Figure 3B). The mean latency of the first emetic episode under this treatment increased significantly to 12.81 ± 4.03 hours (range 6.67 to 32.35). Compared with control values, the cumulative severity (28.5 ± 5.16, range 15 to 51) remained unchanged.

Repetitive administration

In seven other piglets, we administered an initial dose (2 mg/kg) of BIMU 1, followed by 2 mg/kg every 5 hours for 30 hours (i.e. a cumulative dose of 14 mg/kg; Figure 3C). Statistical evaluations were performed as described above.

Following this protocol, BIMU 1 significantly increased the latency of the first emetic event to 35.01 ± 7.88 (range 9.53 to 60). However, the cumulative severity (19.14 ± 6.50, range 0 to 49) remained similar to that of control animals. Under this protocol, a complete control (i.e. no vomiting during 60 hours) was achieved in two out of seven piglets.

TEMPORAL CHANGES OF THE GASTROINTESTINAL TISSUE LEVELS OF 5-HT FOLLOWING CISPLATIN ADMINISTRATION

This series of experiments was carried out to measure the tissue concentrations of serotonin in the gastric, duodenal, ileal, and distal colonic mucosa of piglets receiving only hyperhydration (i.e. control animals, n = 6), or hyperhydration followed by cisplatin administration at a dose of 5.5 mg/kg (i.e. experimental animals, n = 15). Experimental animals were sacrificed at various times following cisplatin administration:

i) during the emetic peak of the acute phase (+ 1.5 hours, n = 4)
ii) at the end of the acute phase (+ 16 hours, n = 3)
iii) at the onset of the emetic peak of the delayed phase (+ 21 hours, n = 2)
iv) 40 hours following cisplatin injection (+ 40 hours, n = 3)
v) at the end of the observation period (+ 60 hours, n = 3).

Tissues were removed quickly from the gut of piglets killed by lethal sodium thiopental administration (50 mg/kg, rapid bolus i.v.) and exsanguination. Mucosae

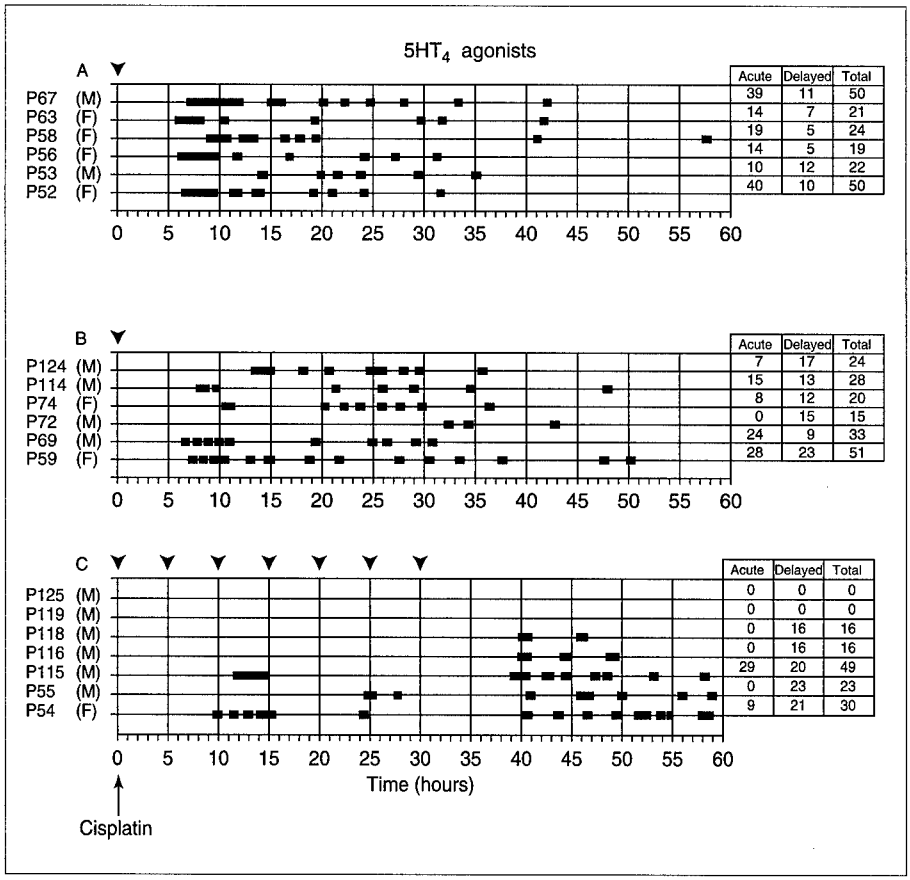

Figure 3. Pattern during the 60-hour observation period of the emetic episodes in piglets treated with a 5-HT_4 receptor agonist.

A and B: Animals receiving a single initial administration of BIMU 1 at the dose of 2 and 4 mg/kg, respectively

C: Animals treated repeatedly (seven injections) with BIMU 1 (2 mg/kg). In each panel, cisplatin (5.5 mg/kg, i.v.) was administered at time zero. Same representation as in Figure 1A and 2. Arrowheads above panels indicate the timing of injections of BIMU 1.

were peeled off with a scapel blade, frozen in liquid nitrogen and stored at –80°C until analysis. On the day of analysis, tissue samples were homogenized with an ultrasonic disruptor, and centrifuged. Serotonin in the supernatant was separated using a reversed-phase column and measured by HPLC with electrochemical detection. The protein concentration of homogenates was determined by a modified Lowry procedure. Results were expressed as ng of serotonin per mg of protein, and statistical evaluations were done using a one-way analysis of variance. Significance was accepted at $p < 0.05$.

The 5-HT tissue concentration of control piglets (i.e. not treated with cisplatin) were 7 ± 1.6, 26 ± 1.7, 5 ± 0.7, and 8 ± 1.2 in the gastric, duodenal, ileal, and distal colonic mucosa, respectively. The concentration of serotonin in the duodenum increased (91 ± 25) significantly during the acute phase (+ 1.5 hours), then decreased but remained higher than the control values at +16 hours (43 ± 3.1), returned to control values (23 ± 2.9) just before the emetic peak of the delayed phase (+ 21 hours), again increased significantly at + 40 hours (49 ± 2.19), and remained at high levels (53 ± 7.4) until the end of the observation period (+ 60 hours). The development of the acute phase of emesis was not correlated with significant changes in 5-HT tissue levels in the ileal (4 ± 0.8) and the distal colonic (7 ± 0.4) mucosa. In contrast, significant changes were observed in the ileal mucosa at +16 hours, + 40 hours and + 60 hours (8 ± 0.7, 13 ± 2.41, and 12 ± 2.6, respectively), and in the distal colonic mucosa at + 60 hours (18 ± 4.5). Serotonin concentrations remained unchanged throughout the chemotherapy course in the gastric mucosa. Results are summarized in Figure 4.

DISCUSSION AND CONCLUSIONS

Most studies of the emetic effect of cytotoxic drugs administered during anti-cancer chemotherapy have focused on the first day following the onset of treatment. Delayed emesis, defined as the less intense but more prolonged bouts of vomiting occuring after the first day and lasting up to 1 week (3), has received minimal attention. Consequently, the mechanisms underlying delayed nausea and vomiting are little understood, and the management of delayed emesis remains less than satisfactory with existing therapies, with around 50% of patients remaining uncontrolled. At the present time, the clear benefit provided by the anti-emetic 'setrons' in the control of emesis during the acute phase contrasts markedly with the doubts about their efficacy during the delayed phase. Thus, the role of serotonin, if any, in the delayed phase remains to be proved.

Our results indicate that the piglet exhibits a high sensitivity to cisplatin without lethality, and is a suitable model in which to study both the acute and delayed phases of emesis. In this animal, the main effect of 5-HT_3 receptor antagonists (i.e. granisetron and ondansetron), administered as single bolus just before cisplatin, is to delay significantly the onset of the acute phase. However, even with high doses (e.g. 7 mg/kg), both 'setrons' appear unable to reduce the incidence of vomiting throughout the chemotherapy course. This could result from either the low affinity of 5-HT_3 receptors in the piglet (see Chapter 9 for a discussion of this issue), or to rapid metabolism of these two compounds in this species. The second hypothesis is more likely, as it is supported by the results obtained with multiple dosing. The total incidence of vomiting (i.e. cumulative severity) was significantly reduced only when piglets were repeatedly administered granisetron at the dose of 1 mg/kg (i.e. a cumulative dose of 7 mg/kg). The most striking observation was the complete

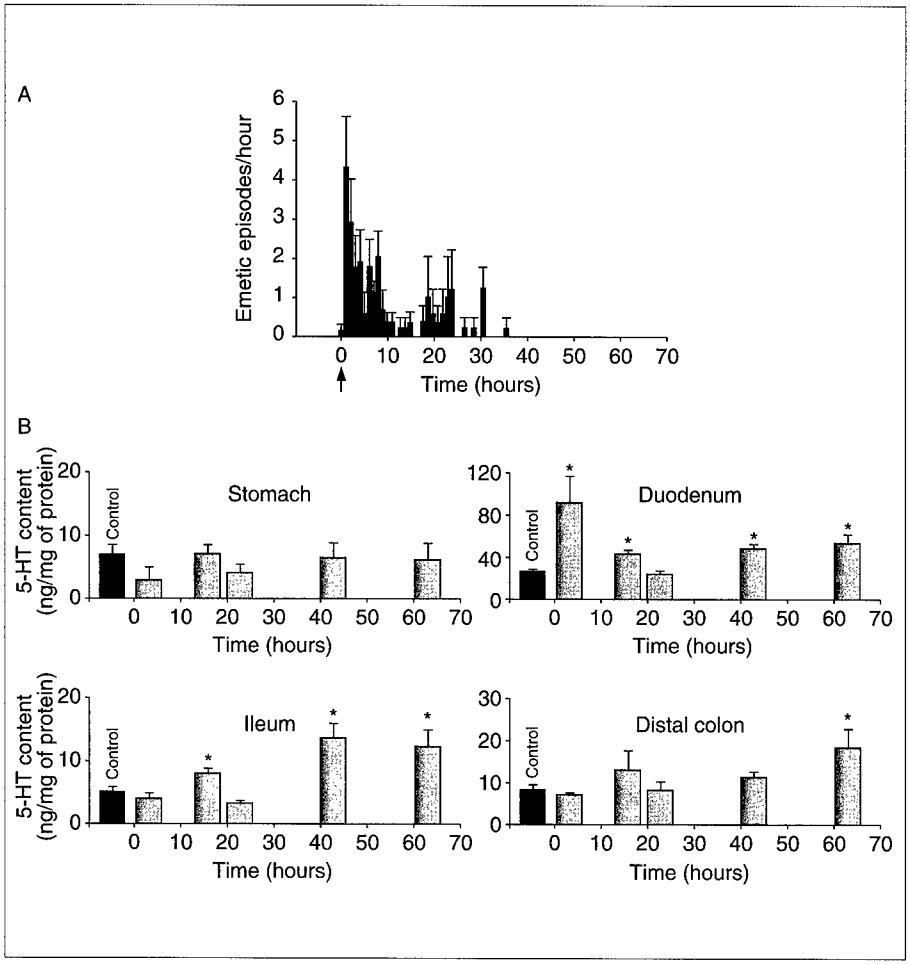

Figure 4. A: Distribution of the emetic episodes exhibited by piglets (n = 15) used to determine the gastrointestinal tissue levels of 5-HT after cisplatin dosing (5.5 mg/kg i.v., at T = 0, arrow). Same representation as Figure 1B.

B: Temporal cisplatin-induced changes of the concentrations of 5-HT in gastric, duodenal, ileal and distal colonic mucosae. The gastrointestinal tissue levels of 5-HT were measured during:

i) the emetic peak of the acute phase (+1.5 hours; n = 4);

ii) the transition between the acute and delayed phases (+16 hours; n = 3);

iii) the onset of the delayed phase (+21 hours; n = 2);

iv) during the delayed phase, that is, 40 (n = 3) and 60 (n = 3) hours following cisplatin administration.

Results expressed as mean ± SEM. *Significant changes at $p < 0.01$ compared with controls (n = 6–8).

control of both the acute and delayed phases in three out of eight piglets. In addition, the five remaining animals that vomited during this protocol exhibited their first emetic episode with a latency of 34.68 ± 6.5 hours. This demonstrates that the onset of the delayed phase that normally begins at 18 hours following cisplatin injection can be delayed greatly by 5-HT_3 receptor antagonists. Repetitive administrations (i.e. same protocol as with 'setrons') of BIMU 1 (a 5-HT_4 receptor agonist) at the dose of 2 mg/kg (i.e. a total dose of 14 mg/kg) also resulted in a complete control of vomiting in two out of seven piglets. Recently, Gebauer and co-workers (9) have demonstrated the existence of 5-HT autoreceptors on the enterochromaffin cells of the guinea-pig, an animal that does not vomit. They concluded that activation of a 5-HT_4 autoreceptor suppresses the release of 5-HT, whereas activation of the 5-HT_3 autoreceptor enhances the release. Demonstration of the existence of such a 5-HT_4 autoreceptor on enterochromaffin cells in the piglet would provide an explanation for the anti-emetic effects observed with BIMU 1 in our experiments.

In conclusion, the 5-HT_3 receptor antagonist granisetron and the 5-HT_4 receptor agonist BIMU 1 can provide complete control of cisplatin-induced emesis when administered during both the acute and the delayed phases. In addition, the tissue concentrations of serotonin in the duodenal mucosa increase rapidly at the onset of the acute phase, then return to control values at the end of this phase, and increase again during the delayed phase. All these results support the hypothesis of a role for serotonin in the delayed phase of vomiting. Further investigations are required to verify this hypothesis, and to determine the mechanisms underlying nausea and vomiting during the delayed phase.

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Chapter 20

5-HT_4 receptor involvement in emesis

M. Tonini

INTRODUCTION

A great deal of progress has been made in the last few years in the understanding of the role played by serotonin (5-hydroxytryptamine or 5-HT) in several physiological and pathophysiological conditions, both in the central nervous system (CNS) and in the periphery (1–3). The identification of multiple 5-HT receptors and the availability of selective 5-HT receptor agonists and antagonists have been primarily responsible for the rapid progress in this field. Out of the seven recognized classes of 5-HT receptors (5-HT_1–5-HT_7), those involved in radiation- or chemotherapy-induced emesis belong principally to the 5-HT_3 class (4), although recently it has been claimed that 5-HT_4 receptors participate in this process ((5); see also Chapter 19).

Peripherally, 5-HT_3 receptors (6), which are ligand-gated ion channels, are located on enteric neuronal membranes, on cell bodies of myenteric and submucous plexus neurones, on sympathetic nerves, on vagal abdominal sensory endings (i.e. the main site for the anti-emetic action of 5-HT_3 receptor blockers), on vagus nerve afferent fibres (including the cell bodies in the nodose ganglion), and on enterochromaffin cells in the mucosa of the upper gastrointestinal tract. In neural tissues, their stimulation is associated with fast, transient membrane depolarization and transmitter release. In enterochromaffin cells, 5-HT_3 receptors enhance 5-HT release via a positive feed-back mechanism ((7); see also Chapter 7). Both mechanisms are probably involved in chemotherapy-induced emesis (see below).

5-HT_4 receptors (8), which are positively coupled to adenylate cyclase, were first characterized in cell cultures of mouse embryo colliculi. The pharmacological profile of these receptors was subsequently found to be similar to that of 5-HT receptors located in other areas of the CNS and, peripherally, in the alimentary tract, the heart, the urinary tract and the adrenal glands. In the gastrointestinal tract, neural and non-neural 5-HT_4 receptors have been demonstrated. Neural receptors, which are located on excitatory pathways where they evoke transmitter release, are those involved in the gastrointestinal prokinetic action of benzamide (cisapride, renzapride, zacopride) and benzimidazolone derivatives (BIMU 1, BIMU 8). Non-neural enteric 5-HT_4 receptors are present in oesophageal smooth muscle cells and are inhibitory in nature. It is interesting to point out that all the above compounds, beside acting as agonists at 5-HT_4 receptors, also possess antagonist

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Table 1. Interaction profile of benzamide and benzimidazolone derivatives with 5-HT_3 and 5-HT_4 receptors.

Chemical class	5-HT_3 receptors	5-HT_4 receptors
Benzamides		
Metoclopramide*	Low affinity antagonist	Partial agonist
Cisapride	Low/moderate affinity antagonist	Full/partial agonist
Renzapride	Moderate/high affinity antagonist	Full agonist
Zacopride	High affinity antagonist	Full/partial agonist
Benzimidazolones		
BIMU 1	High affinity antagonist	Partial agonist
BIMU 8	High affinity antagonist	Full agonist
DAU 6215 (Itasetron)	High affinity antagonist	Partial agonist/ silent antagonist

*Also endowed with antagonist properties at dopamine D_2 receptors

properties (though with different potencies) at 5-HT_3 receptors (Table 1). At least three of the aforementioned compounds (i.e. renzapride, zacopride and BIMU 1) have been found to protect experimental animals (ferrets and dogs) from chemotherapy-induced emesis. Zacopride, on the other hand, although it inhibits cisplatin-induced emesis, is emetic when given alone. This latter effect was originally ascribed to 5-HT_4 receptor stimulation (5).

Based on the improved knowledge of the mechanisms whereby 5-HT is released from enterochromaffin cells (7), and of the role of 5-HT in emesis and gastrointestinal motility (9, 10), the effects of mixed 5-HT_4 receptor agonists/ 5-HT_3 receptor antagonists on chemotherapy-induced emesis and associated gut motor disturbances will be discussed.

CHEMOTHERAPY-INDUCED EMESIS

The role of 5-HT_3 receptors

Among the toxic effects induced by chemotherapeutic agents, nausea and vomiting have been reported as the most distressing events and a risk for therapy discontinuation. The introduction of selective antagonists at 5-HT_3 receptors into clinical practice led to a spectacular improvement in the compliance of patients undergoing chemotherapy. In fact, compounds like ondansetron, granisetron and tropisetron are effective against nausea and vomiting induced by cisplatin and other emetogenic cytotoxic drugs, and are devoid of the typical side-effects (in particular extrapyramidal effects) of anti-emetics with anti-dopaminergic properties, such as metoclopramide (11). The rationale for the use of 5-HT_3 receptor antagonists in the control of emesis stems from evidence that chemotherapy and radiation promote the release of endogenous mediators, like 5-HT, from intestinal mucosal cells (Figure 1).

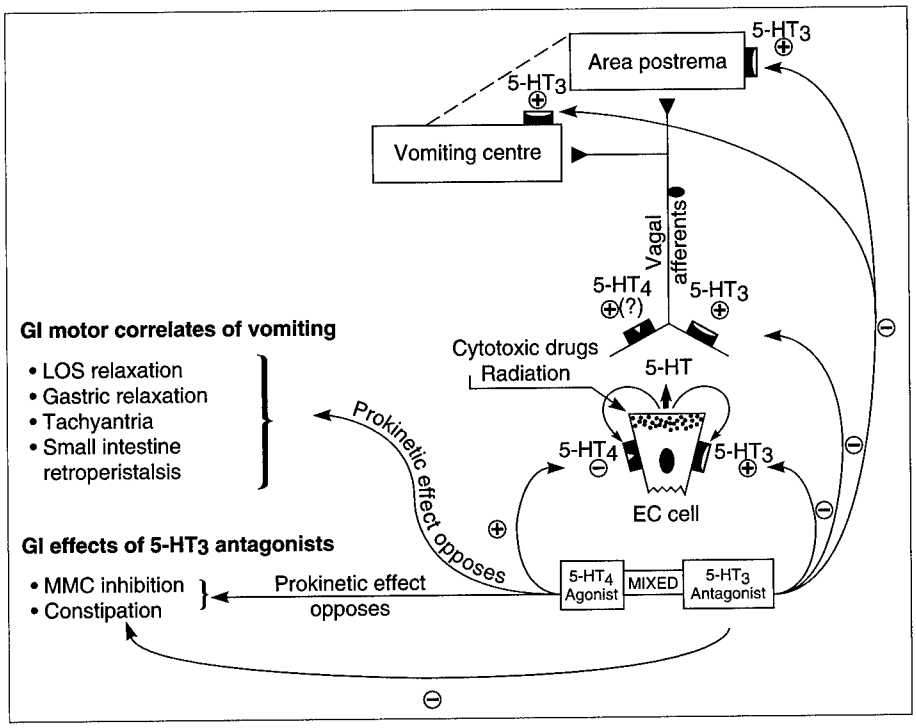

Figure 1. Possible sites of action of mixed 5-HT_4 receptor agonists/5-HT_3 receptor antagonists as anti-emetics in chemotherapy- or radiation-induced emesis. Like pure 5-HT_3 receptor antagonists, mixed 5-HT_4 receptor agonist/5-HT_3 receptor antagonist anti-emetics block 5-HT_3 receptors on enterochromaffin (EC) cells, peripheral and central terminals of vagal afferents, and in the CNS (vomiting centre), which are classical targets for anti-emetic action. Concomitantly, the activation of 5-HT_4 receptors may contribute to further inhibition of 5-HT release from enterochromaffin cells. A prokinetic effect resulting from 5-HT_4 receptor activation may counteract gastrointestinal correlates of vomiting (lower oesophageal sphincter and gastric relaxation, tachyantria, and small intestine retroperistalsis) and intestinal side-effects produced by 5-HT_3 receptor antagonism, that is, intestinal migrating motor complex inhibition and colonic constipation.
LOS, lower oesophageal sphincter; EC cell, enterochromaffin cell; MMC, migrating motor complex.

By acting on 5-HT_3 receptors located on sensory nerve endings, released 5-HT activates abdominal vagal afferent fibres that project to the area postrema and the vomiting centre, and trigger the vomiting reflex (4). In this respect, it is well documented that cisplatin-induced emesis is accompanied by an increase in plasma 5-HT levels and in urinary concentrations of 5-hydroxyindolacetic acid (5-HIAA), the main 5-HT metabolite (12, 13). In addition, this type of vomiting is blocked by

sectioning the visceral afferent nerves. Although blockade of peripheral 5-HT_3 receptors is the main mechanism leading to inhibition of cisplatin-induced emesis, blockade of 5-HT_3 receptors in the dorsal vagal complex may also contribute to the anti-emetic effect (14). Recent evidence, however, indicates that those 5-HT_3 receptors that were originally thought to be located in the area postrema are located on the central terminals of vagal afferent fibres (Figure 1), which project to this area (see Chapter 11). These 'central' sites could be an additional target for any circulating 5-HT_3 receptor antagonist, independent of its ability to enter the CNS, as the area postrema is outside the blood-brain barrier.

The role of 5-HT_4 receptors

The fact that some anti-emetics acting as 5-HT_3 receptor antagonists may also activate 5-HT_4 receptors raises the question of whether the latter sites may be involved in nausea and vomiting. Assessment of the role of 5-HT_4 receptors has been complicated by the finding that zacopride, a mixed 5-HT_4 receptor agonist/5-HT_3 receptor antagonist, although it inhibits (via i.v. or i.p.) cisplatin-induced emesis, is, paradoxically, emetogenic by itself, especially when administered orally in ferrets (15–17). As no other 5-HT_3 receptor antagonist has been reported to induce emesis in ferrets, this emetic response to zacopride might be related to agonism at 5-HT_4 receptors. A preliminary report supported this hypothesis, although the 5-HT_4 receptor antagonist tested against zacopride-induced emesis (tropisetron: a mixed 5-HT_3/5-HT_4 receptor antagonist with high affinity for the former and low affinity for the latter sites) was not adequately selective (5). In addition, vagal fibres, which play a pivotal role in the transmission of emetogenic stimuli to the CNS, have been shown to be depolarized not only by 5-HT_3, but also by 5-HT_4 receptor stimulation (18). Unlike 5-HT_3 receptor-induced depolarization, the depolarization evoked in the rat isolated vagus nerve by 5-HT_4 receptor stimulation was small and prolonged. For example, renzapride, whose affinity for 5-HT_4 receptors is 5–10-times as high as that of zacopride in both the CNS and intestine (8), causes a maximum depolarizing response of only 26 μV. In comparison, the selective 5-HT_3 receptor agonist 2-methyl-5-HT caused a depolarizing response of 410 μV. Based on this evidence, the zacopride-activated 5-HT_4 receptor component of the vagally mediated emesis in the ferret seems to be negligible. Furthermore, other observations argue against the hypothesis that 5-HT_4 receptors participate in the emetic response to zacopride. First, of the two enantiomers of zacopride, the S-form, which is predominantly responsible for emesis, behaves, like 2-methyl-5-HT or phenylbiguanide, as a 5-HT_3 receptor agonist in the ferret and may therefore induce vomiting through this mechanism (16, 17). Second, other 5-HT_4 receptor agonists, such as cisapride, renzapride or BIMU compounds, have never been found to induce nausea or vomiting. Very recently, two separate studies provide good evidence that 5-HT_4 receptors are not involved in emesis. Unlike 2-methyl-5-HT, the selective 5-HT_4 receptor agonist 5-methoxytryptamine failed to enhance the discharge of vagal mucosal afferent fibres in the rat *in vivo* (D. Grundy unpublished observations), and

most notably, the potent and selective 5-HT_4 receptor antagonist GR 125487 failed to prevent zacopride-induced emesis in the ferret (see Twissell *et al.*, P4, p.246).

ANTI-EMETIC EFFECT OF DRUGS WITH MIXED 5-HT_4 RECEPTOR AGONIST/5-HT_3 RECEPTOR ANTAGONIST ACTION

Rather than inducing vomiting, some benzimidazolone derivatives with mixed activity at the two 5-HT receptors (19) have been shown to inhibit cisplatin-induced emesis with a potency greater than that expected from their antagonism at 5-HT_3 receptors (10, 20). For example, BIMU 1, which displays, both *in vitro* and *in vivo* affinity for 5-HT_3 sites comparable to that of the more selective 5-HT_3 receptor antagonist ondansetron (Table 2), is 10-times more potent than the latter in inhibiting cisplatin-induced emesis in the dog (Table 3). The same also applies to zacopride, which is 10-times more potent than ondansetron and tropisetron in the same species (21), despite the fact that the affinity of zacopride for the 5-HT_3 receptor is similar to that of the other two 5-HT_3 receptor antagonists. Although the potent anti-emetic effect of BIMU 1 and zacopride in dogs might also be ascribed to a potentially favourable kinetic profile, the above findings suggest that the simultaneous blockade of 5-HT_3 receptors and stimulation of 5-HT_4 receptors may contribute to the observed effect. This assumption is supported by recent *in vitro* findings indicating that 5-HT may modulate its own secretion via 5-HT_3 and 5-HT_4 sites, the former mediating stimulation, and the latter mediating inhibition of 5-HT release (Figure 1) ((22); see also Chapter 9). In fact, both 5-HT_3 receptor antagonists and 5-HT_4 receptor agonists have been found to inhibit the secretion of 5-HT from the enterochromaffin cells. If modulation of 5-HT secretion is a relevant, additional target for anti-emetic action, drugs interfering with this process should display higher clinical efficacy. In particular, compounds that combine potent antagonism at 5-HT_3 receptors and agonism at 5-HT_4 receptors are expected to protect more effectively against cytotoxic drug-induced vomiting, as observed with BIMU 1 and zacopride in the dog. This hypothesis would be further substantiated by proving that concomitant administration of 5-HT_3 receptor antagonists (at submaximal doses) and 5-HT_4 receptor agonists has higher anti-emetic efficacy than 5-HT_3 receptor antagonists alone.

Inhibition of gastrointestinal propulsion, leading to constipation, has been reported in patients treated with 5-HT_3 receptor antagonists (11). The pathophysiological basis of this event may be blockade of neural 5-HT_3 receptors that mediate fast excitatory transmission in enteric plexuses, both in ascending and descending pathways involved in peristalsis. On the other hand, it is well known that 5-HT_4 receptor agonism is associated with the activation of intestinal cholinergic pathways, a mechanism that is considered to be responsible for the gastrointestinal prokinetic action of benzamide and benzimidazolone derivatives (9). The same mechanism might counteract the lower oesophageal sphincter and gastric relaxation,

Table 2. *In vitro* and *in vivo* interactions of BIMU 1 and ondansetron with 5-HT_3 receptors.

Compound	Receptor binding affinity in rat cerebral cortex [pK_i]	Antagonism of 5-HT induced tachycardia in rabbit heart [pA_2]	Antagonism of 5-HT-induced Bezold-Jarisch reflex in rats (Vagal reflex bradycardia) [ID_{50} (-log mol/kg)]
BIMU 1	8.67	10.42	8.28
Ondansetron	8.42	10.38	8.14

Adapted from (19).

Table 3. Inhibition of cisplatin-induced emetic episodes (retching and vomiting) in the dog by three anti-emetic agents.

Compound	ID_{50} (nmol/kg i.v.)
BIMU 1	12
Ondansetron	140
Metoclopramide	2200

Adapted from (10) and (20).

as well as small intestine retroperistalsis, which are well known components of the emetic reflex (4), and the inhibitory action of 5-HT_3 receptor antagonism on intestinal propulsion. Therefore, anti-emetics with mixed 5-HT_4 receptor agonist/ 5-HT_3 receptor antagonist properties could theoretically produce three major benefits (Figure 1):

- improved relief of chemotherapy-induced nausea and vomiting, by inhibition of 5-HT secretion from enterochromaffin cells and blockade of intestinal vagal afferent activation
- relief of perturbed motility in the upper gastrointestinal tract, by reinstating normal peristalsis
- lack of constipation, which represents a frequent side-effect of pure 5-HT_3 receptor antagonists, although the latter adverse event has never been reported to decrease compliance in patients receiving anti-emetic treatment.

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Chapter 21

Are 5-HT_3 receptor effects on gastrointestinal motility relevant to anti-emesis?

J. A. J. Schuurkes

INTRODUCTION

5-HT_3 receptor antagonists are effective in the treatment of emesis caused by chemotherapy or irradiation. Early 5-HT_3 receptor antagonists such as metoclopramide and dazopride also possess gastroprokinetic activity (via 5-HT_4 receptor agonism) which in the past led to the hypothesis that the anti-emetic effects of these compounds could contribute to their motility-stimulating properties (1). Later on, more potent and selective 5-HT_3 receptor antagonists (e.g. ondansetron, tropisetron, granisetron) devoid of prokinetic effects became available, contradicting the hypothesis. However, as recent observations have indicated that these selective 5-HT_3 receptor antagonists are not completely devoid of effects on gastrointestinal motility and/or secretion, the discussion on the role of their motility effects in anti-emesis was refuelled. The aim of this review is therefore to try to answer the question of whether the motility effects of selective 5-HT_3 receptor antagonists could contribute to their anti-emetic efficacy. To answer this question, the effects of 5-HT_3 receptor antagonists on the motility correlates of the emetic reflex or on other motility parameters not directly involved in the emetic reflex need to be determined. Table 1 lists the motility parameters involved in the emetic reflex, the changes

Table 1. Gastrointestinal motility parameters, the emetic reflex and 5-HT_3 receptor antagonists.

Motility parameters	During the emetic reflex	Effect of 5-HT_3 receptor antagonists
UOS	Reduced	?
LOS	Reduced	Moderately increased
Gastric tone	Reduced	No effect (reduced)
Antral slow wave frequency	Increased	?
Gastric emptying	Delayed	No effect (accelerated)
Occurrence of Phase III of the MMC	Reduced	Reduced
Number of retrograde giant contractions	Increased	?
Colonic transit (GMC)	Accelerated	Delayed

UOS, Upper oesophageal sphincter; LOS, lower oesophageal sphincter; MMC, migrating motor complex; GMC, giant migrating contractions.

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occurring during the reflex, and the effects of 5-HT_3 receptor antagonists on these parameters. Based on these data, an attempt is made to answer the question of whether the reported effects might contribute to the anti-emetic efficacy of 5-HT_3 antagonists.

OESOPHAGEAL SPHINCTERS AND GASTRIC TONE

The upper oesophageal sphincter (UOS) relaxes during emesis (2). No data are available on the effects of 5-HT_3 receptor antagonists on the UOS.

The lower oesophageal sphincter (LOS) also relaxes during the emetic reflex (2). Tropisetron slightly enhances pressure in the LOS in volunteers (3), in whom a mean maximal increase of 4 mmHg was observed. This moderate increase is not likely to form a major contribution to the anti-emetic efficacy of 5-HT_3 receptor antagonists. Data obtained in fasted anaesthetized ferrets suggest a possible role for 5-HT_3 receptor antagonists in the reduction of LOS relaxations induced by balloon-distension of the gastric corpus (4). Whether this observation can be extrapolated to a reduction in number and/or size of relaxations of the LOS during the emetic reflex remains to be studied.

During emesis, gastric tone is reduced as the stomach wall relaxes (2). Serotonin (5-hydroxytryptamine, 5-HT) can induce such a relaxation. We were interested, therefore, to determine the effects of 5-HT_3 receptor antagonists on 5-HT-induced relaxations of an isolated preparation of the guinea-pig stomach. Both granisetron and ondansetron did not affect gastric relaxations induced by intra-arterial administration of 5-HT (Figure 1A). This is in good agreement with our observations in dogs. In conscious dogs equipped with a gastric cannula, pressure–volume curves were determined with a barostat. Pressure steps of 2 mmHg were chosen, ranging from 2 to 14 mmHg. Ondansetron or granisetron (both at 0.16 mg/kg s.c.) did not affect gastric compliance (Figure 1B). In the anaesthetized rat, granisetron and ondansetron even enhanced gastric relaxations induced by vagus nerve stimulation (5). Therefore, the available data do not suggest that effects on gastric tone could contribute to the anti-emetic properties of 5-HT_3 receptor antagonists.

ANTRAL SLOW WAVE FREQUENCY AND GASTRIC EMPTYING

Antral slow wave frequency is increased during the emetic reflex (2). Observations on the effect of 5-HT_3 receptor antagonists on antral slow wave frequency have not been reported.

In rats, 5-HT_3 receptor antagonists are effective in accelerating gastric emptying of a test meal (6, 7). Granisetron and ondansetron can completely reverse the inhibitory effect of cisplatin on gastric emptying of a semi-solid acaloric test meal

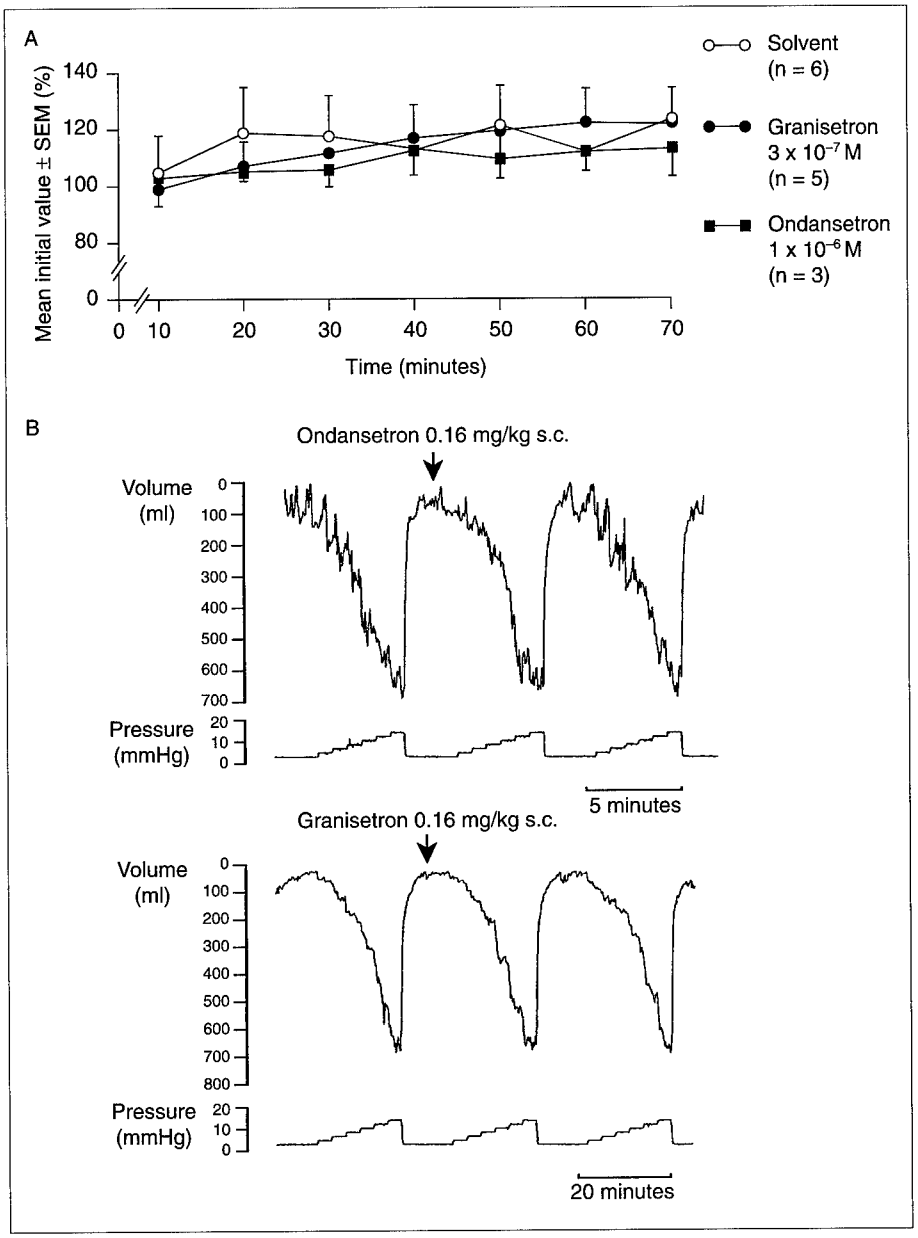

Figure 1. A: 5-HT_3 receptor antagonists do not inhibit 5-HT-induced gastric relaxations in the isolated guinea-pig stomach (5-HT 7×10^{-9} M in 0.1 ml injected via the left gastric artery, in the presence of atropine 3×10^{-7} M in the bath).
B: 5-HT_3 receptor antagonists do not affect gastric compliance measured with a barostat in a conscious dog.

(8). This might be caused by a pure functional antagonism or it may be a reflection of the anti-emetic properties of the 5-HT_3 receptor antagonists interrupting a correlate of emesis in the rat, which is characterized and limited to a delay in gastric emptying. These effects of 5-HT_3 receptor antagonists to accelerate gastric emptying are restricted to the rat, as similar effects were not observed in other species, including humans. Tropisetron and granisetron at a dose of 0.63 mg/kg p.o. did not affect gastric emptying of a liquid acaloric test meal, delayed by the administration of the α_2 receptor agonist lidamidine (0.63 mg/kg s.c.) (Schuurkes, unpublished data). In human volunteers, tropisetron slightly accelerated gastric emptying (9) or had no effect (10). Similarly, ondansetron did not affect gastric emptying of a caloric mixed test meal (11). In anorexia patients (12), tropisetron also did not influence gastric emptying.

Therefore, it seems unlikely that an effect of 5-HT_3 receptor antagonists on gastric emptying could contribute to their anti-emetic effect.

OCCURRENCE OF PHASE III OF THE MIGRATING MOTOR COMPLEX (MMC)

Cisplatin administration to conscious dogs leads to an interruption of the cyclic rises in plasma motilin levels and prevents the occurrence of phase III activity of the migrating motor complex (MMC) (13). Unfortunately, no data are available on the motor patterns in fasted dogs in the presence of cisplatin after pretreatment with 5-HT_3 receptor antagonists. As 5-HT_3 receptor antagonists have also been demonstrated to prevent the occurrence of phase III activity on the stomach in dogs and human volunteers (14, 15) it seems unlikely that this inhibitory effect could contribute to the anti-emetic properties of 5-HT_3 receptor antagonists. On the other hand, in individual patients in which phase III-like activity would still be present during chemotherapy or irradiation, its inhibition might help to reduce the initiation of the emetic reflex. This possibility was illustrated by Wingate *et al.* (16), who demonstrated a coupling between the occurrence of phase III activity in the upper gastrointestinal tract and episodes of vomiting in a fasting patient.

The effects of 5-HT_3 receptor antagonists on motor patterns in fasted dogs and volunteers (14, 15) are thought to be mediated via inhibition of the effects of endogenous motilin as an inducer of gastric MMC activity. Indeed, in conscious dogs 5-HT_3 receptor antagonists will also inhibit the effects of exogenous motilin or the effects of the motilin agonist EM523 (an erythromycin analogue) both *in vitro* (17, 18) as well as *in vivo* (19). In dogs, 5-HT_3 receptor antagonists (e.g. granisetron) do not affect cyclic plasma motilin levels (Figure 2A) (14), suggesting that in this species motilin induces phase III activity in the stomach via the release of 5-HT acting on 5-HT_3 receptors. This is in contrast to data in volunteers, where ondansetron has been shown to prevent cyclic motilin changes and is unable to inhibit phase III-like contractions induced by erythromycin itself

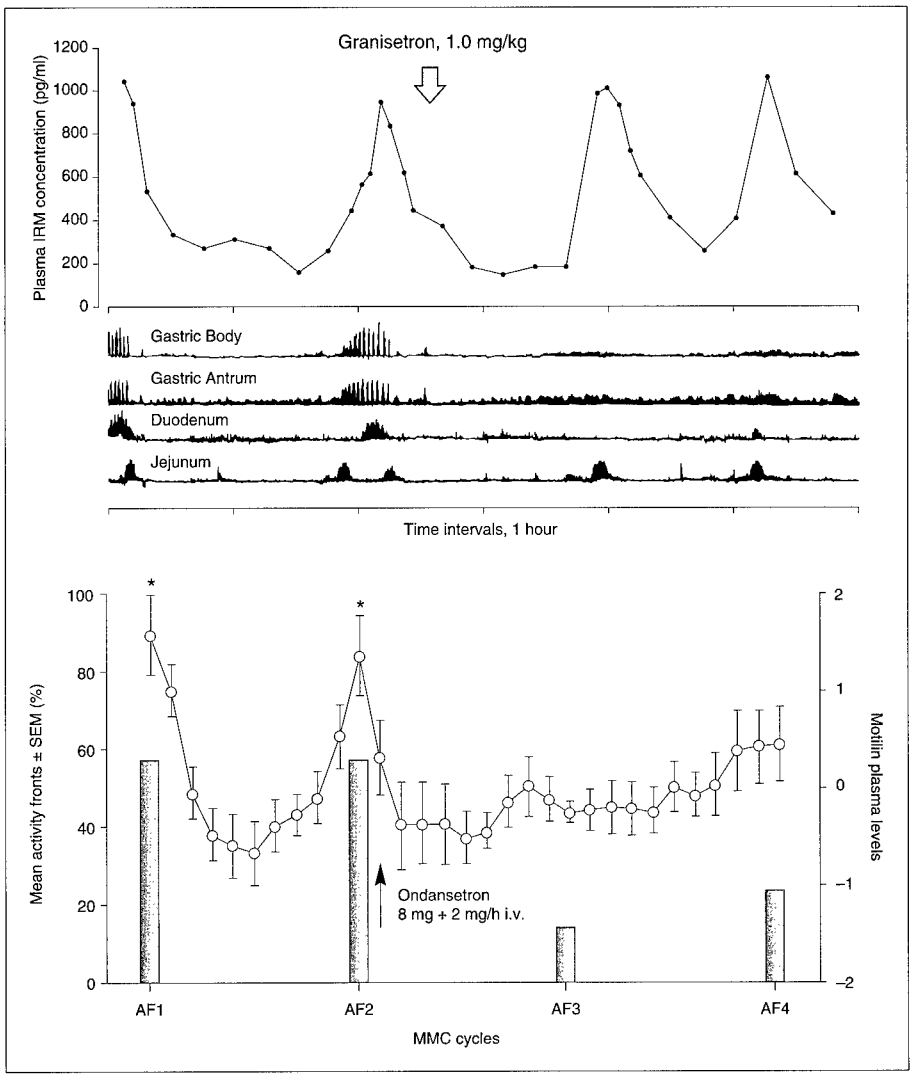

Figure 2. A: Granisetron prevents gastric migrating motor complex activity, but does not affect cyclic changes in plasma motilin levels in a conscious dog. Reproduced with permission from (14).

B: Ondansetron reduces the occurrence of gastric activity fronts and prevents a rise in plasma motilin levels in healthy volunteers. Reproduced with permission from (15). MMC, migrating motor complex.

(Figure 2B) (15), leading to the hypothesis that in human volunteers endogenous 5-HT, via an action on 5-HT_3 receptors, is involved in the release of motilin, which consequently could initiate gastric phase III activity. Whether these

discrepancies reflect a true species difference in the control of fasted motility or whether this reflects a difference between the 5-HT_3 receptor antagonists used (in humans, only data on ondansetron are available, whereas in dogs plasma motilin levels were only measured after granisetron) remains to be determined.

NUMBER OF RETROGRADE GIANT CONTRACTIONS

Retrograde giant contractions constitute an important part of the emetic reflex, bringing intestinal contents back into the relaxed stomach (2). The occurrence of retrograde giant contractions or a burst of retrograde phasic contractions (20) often precedes vomiting, but vomiting itself cannot be prevented by blocking the retrograde contractions. For instance, retrograde peristalsis induced by apomorphine in conscious dogs can be blocked by vagotomy, but this blockade does not prevent vomiting (21). Unfortunately, the effects of 5-HT_3 receptor antagonists on retrograde motor activity have not been reported, although their incidence increases in the small intestine of dogs exposed to radiation, as does the incidence of giant migrating contractions (22, 23).

COLONIC TRANSIT

During the emetic reflex induced by irradiation, the number of giant migrating contractions on the large bowel is enhanced (22, 24), suggesting accelerated transit through the colon. The effect of chemotherapy on the incidence of giant migrating contractions appears not to have been studied, although it is likely that they will increase as with radiation. Although the effects of 5-HT_3 receptor antagonists on giant migrating contractions are unknown, they may reduce this side-effect of cancer therapy through their delaying effect on colonic transit. This has been demonstrated with different 5-HT_3 receptor antagonists, both in volunteers and in patients with diarrhoea-predominant irritable bowel syndrome (11, 25). A direct relation with the anti-emetic effects of 5-HT_3 receptor antagonism seems unlikely.

CONCLUSION

Table 1 summarized the motility effects occurring during the emetic reflex and the effects of 5-HT_3 receptor antagonists on these parameters. Unfortunately, the quantity of data available to allow a solid conclusion on a possible role for the motility effects of 5-HT_3 receptor antagonists in emesis is rather limited. This may reflect the general feeling, based on negative findings in the available studies, that a contribution by these motility effects is highly unlikely.

1. During the emetic reflex, the upper part of the gastrointestinal tract relaxes and, as a consequence, gastric emptying is delayed. As the parameters of upper gut

motility are not affected by 5-HT_3 receptor antagonists, it is unlikely that they could contribute to the anti-emetic effects of these compounds.

2. The inhibition of phase III of the migrating motor complex is perhaps the most striking motor effect of the 5-HT_3 receptor antagonists. However, as phase III activity might already be reduced during cancer therapy, the contribution of this effect to anti-emesis remains uncertain.

3. As the effects of 5-HT_3 receptor antagonists on retrograde motor activity and on giant migrating contractions have not been studied, their role in emesis cannot be established. The delaying effects on colonic transit may contribute to reduce some symptoms by functionally antagonizing the side-effects of cancer therapy on large bowel transit.

Thus, it seems unlikely that inhibition of the motor correlates of the emetic reflex by 5-HT_3 receptor antagonists contribute to their anti-emetic effects.

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Chapter 22

Opioid receptor involvement in emesis and anti-emesis

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INTRODUCTION

Opioid receptor involvement in the modulation of the emetic reflex is complex and poorly understood. Our current knowledge has been based predominantly on the use of opioid receptor agonists and antagonists which have poor selectivity for the numerous opioid receptor subtypes that are now known to exist. Furthermore, the neurotransmitter systems that opioid drugs modulate to induce and antagonize emesis are probably manifold. This chapter attempts to indicate the mechanisms that may be involved in the emetic and anti-emetic effects of opioid receptor ligands, and how endogenous opioids may integrate with other neurotransmitter substances to regulate emesis in man.

OPIOID RECEPTORS MEDIATING EMESIS AND ANTI-EMESIS

It is difficult to identify with absolute confidence which opioid receptor subtypes are responsible for mediating the emetic and anti-emetic effects of opioid receptor drugs, as the receptor selectivity of such compounds is low. For example, the peptidergic opioid receptor ligands, such as leu-enkephalin, met-enkephalin and beta-endorphin, tested for emetic potential, only exhibit approximately 10-fold affinity between μ and ∂ receptors, and have approximately 25-fold less affinity for κ opioid receptors (1, 2). Therefore, for the purposes of this chapter, the concept of selectivity that will be discussed for some compounds shall only reliably indicate a 'preference' of a compound for the μ/∂ or ∂/μ opioid receptors, where the selectivity is considered to be less than approximately 30-fold at the individual receptors. In particular, some of the κ opioid receptor agonists used in emesis studies are also non-selective for opioid receptor subtypes and therefore their effects are discussed with caution (Table 1).

EMETIC SENSITIVITY OF HUMANS TO OPIOIDS

Morphine and related analgesic opioid agonists are invaluable drugs for the treatment of pain. However, it is certain that opioid drugs (agonists and antagonists)

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Table 1. The binding selectivity profile of some of the opioid drugs used in emesis studies.

Ligand	ki (nM)		
	μ	∂	κ
Met-enkephalin	8.0	4.4	835
Leu-enkephalin	20	3.0	835
Beta-endorphin	2.0	2.7	57
DALA	4.0	1.6	500
DAGO	1.9	345	6090
DSLET	39	1.8	6040
Morphine	0.4	50	100
Fentanyl	0.3	200	218
Sufentanil	1.6	23	125
Ethylketocyclazocine	1.0	3.2	16.0
Tifluadom	7.7	111.2	0.08
Bremazocine	0.62	0.72	0.41
Naloxone	1.78	27.0	17.2
Naltrexone	1.08	6.6	8.5
MR 2266	1.37	6.0	0.69
ICI 154, 129	10, 100	778	> 50, 000

Table modified from (1, 2)

have the potential to induce, or contribute to nausea and vomiting in humans when administered either as single agents, or as adjuncts to anaesthesia (e.g. premedication with opioid drugs is often associated with an increased incidence of postoperative nausea and vomiting (PONV) (3)). Nevertheless, the usefulness of opioid drugs in the clinical setting generally out-weighs the common side-effects of nausea and vomiting, which in most patients rapidly decrease on repeated treatment. Indeed, the poor control of pain may result in increased nausea and vomiting.

Morphine and other opioid drugs such as codeine are known to induce emesis in man (4). The side-effects may be controlled to some degree with the dopamine receptor antagonists metoclopramide, prochlorperazine, or droperidol (4, 5). However, very little information is available to indicate which opioid receptor subtypes are responsible for mediating emesis. Limited reports indicate that the stable met-enkephalin analogue, F 33-824, is emetic when administered intramuscularly at doses of 1–3 mg/adult (6), which may suggest a role for the ∂/μ receptor in addition to μ opioid receptors.

Activation of opioid receptor mechanisms in humans may also contribute to the emesis observed as a consequence of other drug treatments. For example, beta-endorphin (a ∂/μ opioid receptor-preferring ligand) concentrations are elevated and correlate with nausea and vomiting following provocative-motion-(7), and chemotherapy-induced nausea and vomiting (8), but met-enkephalin concentrations are not elevated during chemotherapy-induced emesis (8).

Opioid drugs can induce emesis when administered orally or parenterally, regardless of whether the route is intranasal, transdermal, oral/transmucosal, intrathecal, subcutaneous, intravenous or epidural (3). It seems difficult to dissociate the beneficial analgesic effects of opioid drugs from the side-effects of nausea and vomiting. However, there are reports that low doses of naloxone (50–100 μg) can be used to reverse the emesis induced by intraspinal opioids without significantly affecting analgesia, which may indicate an action at the area postrema.

Certainly it would be interesting to investigate the actions of quaternary naloxone derivatives, which do not cross the blood-brain barrier, to control PONV or emesis caused by opioids used in the treatment of cancer pain (9), but with the proviso that the area postrema has an ineffective blood-brain barrier.

Conversely, the administration of highly lipid-soluble opioid agonists such as fentanyl and sufentanil may result in a lower incidence of nausea after epidural opioid administration, presumably as a function of a more rapid or selective penetration to the vomiting centre to suppress emesis (Figure 1). It is also intriguing that partial agonists such as nalbuphine and butorphanol can reverse the emetic effects of epidural morphine without reversing analgesia (3). Similarly, partial agonists display anti-emetic activity in some animal models of emesis (10, 11). Considering the possible inolvement of ∂ opioid receptors in emesis in humans, it would be of interest to examine whether naltrindole, a selective ∂ opioid receptor antagonist, is capable of reducing PONV or preventing the emesis caused by opioid administration, without reversing analgesia.

STUDIES IN THE DOG

Some endogenous opioid receptor ligands and stable analogues, with moderate selectivity for ∂ and μ opioid receptors, are emetic when administered to animals. For example, in the dog, the ∂/μ opioid receptor-preferring ligand, leu-enkephalin, has been demonstrated to excite area postrema neurones (12) and is emetic when injected intravenously (0.05–0.3 mg/kg); the more stable analogue, [D-Ala^2, Met^5]enkephalinamide (DALA) is also emetic when given intravenously, this emesis can be prevented by low doses of naloxone (13, 14). The emesis induced by the intracerebroventricular (i.c.v.) injection of met-enkephalin 0.5–2.5 μg/kg (another ∂/μ opioid receptor-preferring ligand) is also prevented by low doses of naloxone (8 μg/kg i.c.v), and by lesioning the area postrema (15). The emetic effects of these agents therefore seems to suggest a predominant role for the ∂/μ opioid receptor in opioid-drug-induced emesis.

However, morphine (0.3 mg/kg i.v.) also induces emesis in the dog, which is further suggestive of a μ opioid receptor involvement, but Blancquaert and colleagues (14) considered that the emetic effects of morphine in the dog are mediated preferentially by ∂ opioid receptors. Their hypothesis was based on the absence of an emetic effect of fentanyl, a relatively selective and potent μ opioid

receptor agonist. When the data were compared with the data obtained from the cat (11), important species differences were suggested to exist (see below). Blancquaert and co-workers (14) have hypothesized that because morphine has low lipophilicity,

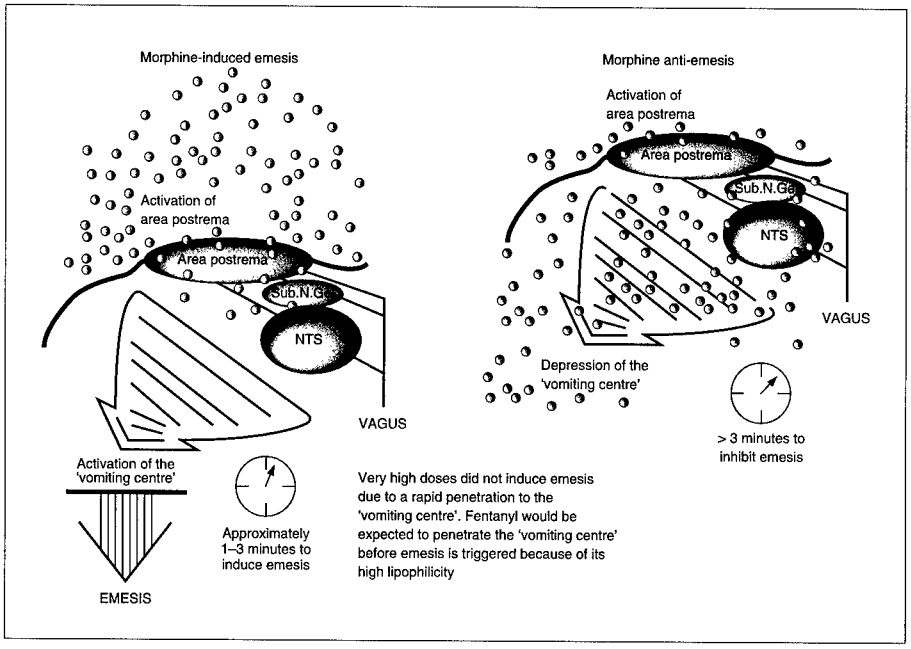

Figure 1. The complex series of experiments described by Costello and Borison (1977) hold the fundamental keys to understanding how opioid drugs have the capacity both to induce and inhibit emesis. Taking morphine as the prototypical opioid receptor agonist, an intracerebroventricular injection (a similar rationale can be used for subcutaneous and intravenous injection) should result in morphine having rapid access to the area postrema, particularly as the area postrema is functionally devoid of an effective blood–brain barrier. Activation of opioid receptors in the area postrema (prevented by naloxone) is hypothesized to result in information being relayed to the 'vomiting centre'. The process of emesis is thus initiated. However, as time progresses, morphine is able to penetrate to the deeper medullary structures to activate opioid receptors (again prevented by naloxone) located in the 'vomiting centre', or at least, close to its final output. Such an action can account for the ability of morphine to prevent its own emetic potential and that to a host of other emetics (i.e. broad-spectrum anti-emetic potential). Certainly, opioid receptors are known to exist in these brain regions (55). Higher doses of morphine are anticipated to reach the anti-emetic site of action at a faster rate to account for the 'bell-shaped' dose–response profile. Compounds that are partial agonists would be expected to be less emetic, and compounds with a high degree of lipophilicity (e.g. fentanyl) would also be expected to be less emetic, as they would rapidly penetrate the 'vomiting centre' to prevent their own emetic action (e.g. behave as pure broad-spectrum anti-emetic agents). NTS, nucleus tractus solitarius; Sub. N. Gel, subnucleus gelatinosus.

high concentrations would build-up at the area postrema to have a predominant effect on ∂ opioid receptors. However, this interpretation does not take account of the fact that low doses of naloxone (a μ opioid receptor-preferring antagonist at low doses) can prevent morphine-induced emesis (14). Further, Hersom and Mackenzie (9) later demonstrated that morphine-induced emesis could be prevented by naltrexone (a non-selective opioid-receptor antagonist), methyl-naloxone (a non-selective opioid receptor antagonist with low blood-brain barrier permeability) and M8008 (a μ/∂ opioid receptor-preferring antagonist).

Evidence for a role of κ receptors in opioid drug-induced emesis in the dog is less clear. The non-selective κ receptor agonists, ethylketocyclazocine (0.03–0.3 mg/kg i.v.; EKC), bremazocine (0.01–0.1 mg/kg i.v.), and tifluadom (1 mg/kg s.c.) are generally not emetic; only EKC-induced emesis in one out of two dogs at the low dose of 0.03 mg/kg (9, 14). Indeed, such agents may inhibit their own emetic action. Future investigations are required using compounds with a greater selectivity for the κ opioid receptor.

STUDIES IN THE CAT

With regard to opioid-induced emesis in the cat, met-enkephalin and leu-enkephalin (∂/μ opioid-preferring receptor agonists) are emetic when administered in doses ranging from 0.1–2.0 mg/cat i.c.v. (10, 16) suggesting a role for ∂/μ opioid receptors in this species. Interestingly, beta-endorphin (a non-selective opioid receptor agonist) is emetic when administered at doses ranging between 0.05–1.00 mg/kg i.v. (17) but not when administered intracerebroventricularly at low doses (0.1 mg/cat) (10). Moreover, Yasnetsov *et al.* (18) found that met-enkephalin, leu-enkephalin, beta-endorphin and [D-Ala^2, D-Leu]enkephalin (DADLE; a ∂/μ opioid-preferring agonist) are emetic when administered intracerebroventricularly over the dose range of 10–300 μg/cat; the emesis to DADLE was prevented by ICI 154,129 (a ∂ opioid-preferring receptor antagonist). Furthermore, morphine can also induce emesis when administered intracerebroventricularly (this is prevented by naloxone but not ICI 154,129 or by area-postrema lesion) (11, 18). Thus, both μ and ∂ opioid receptors appear to be involved in opioid drug-induced emesis in the cat. No studies have addressed the potential of κ opioid receptor agonists to induce emesis in this species.

Some opioid receptor ligands including fentanyl, levorphanol and meperidine have been tested intracerebroventricularly in the cat but have failed to induce emesis (11). Fentanyl also fails to induce emesis when administered peripherally in the dog (14), ferret (19) or *Suncus murinus* (20). However, when the synthetic opioid receptor ligands that normally fail to induce emesis (e.g. fentanyl), or that have an inherently low emetic potential (e.g. methadone and dihydromorphinone), are administered intracerebroventricularly in the cat following a 5mg/kg intraperitoneal or intravenous administration of naloxone (administered as a 1 hour pretreatment),

emesis occurs (11). Similar effects have been reported in *Suncus murinus* with loperamide and naloxone combinations (see below).

STUDIES IN THE FERRET

The ferret, a commonly used species for emesis research, appears to be relatively insensitive to the ∂/μ opioid receptor-preferring ligands leu-enkephalin and met-enkephalin (even at doses up to 1 mg/kg s.c. or i.v. (21)). It is sensitive, however, to [D-Ala^2,NMePhe^4,Gly-ol^5]-enkephalin (DAGO; a selective μ opioid receptor agonist), which induces emesis at doses as low as 0.001–0.030 mg/kg s.c. (21, 22). Furthermore, the non-selective μ/∂ opioid receptor agonists D-Ser, Leu-enkephalin-Thr (DSLET) and DADLE induce emesis at 0.1 mg/kg i.v. (22). Morphine is also capable of inducing emesis at low doses (0.025–2.000 mg/kg s.c. (19, 23, 24)), which would further support a role for μ opioid receptor involvement in emesis in this species. Also, morphine-induced emesis is potently inhibited by naloxone (and quaternary derivatives of naloxone) (23, 25), and by M8008, which tends to confirm a μ/∂ opioid receptor involvement. It is also inhibited by low doses of naltrindole (a ∂ opioid receptor antagonist) (26) and by MR 2266 (a non-selective opioid receptor antagonist with marginal selectivity for the κ opioid receptor) (25). Furthermore U 69593, (a selective κ opioid receptor agonist), is also emetic in ferrets (Rudd, unpublished data). Thus, it appears that all three opioid receptor subtypes (μ, ∂ and κ) may be involved in mediating opioid drug-induced emesis in the ferret.

Loperamide, a μ opioid-preferring agonist, with low lipophilicity induces emesis in the ferret (27). The emesis induced by loperamide can be prevented by naloxonazine (a μ opioid receptor antagonist with some selectivity for the μ_1 opioid receptor), naloxone methyliodide (an opioid receptor antagonist that has poor blood-brain barrier penetration and therefore probably prevents loperamide-induced emesis by acting at the area postrema), and by area postrema lesion. However, if loperamide does activate opioid receptors in the vicinity of the area postrema, they are likely to be on dendrites projecting from the dorsomedial nucleus tractus solitarius (as demonstrated by c-*fos* immunocytochemistry (28)). Furthermore, the opioid receptors stimulated by loperamide are not located on the vagal afferent fibres that terminate from the gastrointestinal tract (28); a similar situation may exist for other opioids to induce emesis.

STUDIES IN *SUNCUS MURINUS*

Suncus murinus is unlike other species commonly used in emesis research, in that it appears to be unresponsive to the emetic effect of morphine (0.1–21.5 mg/kg i.p. or s.c.) or loperamide (0.01–10.00 mg/kg i.p or s.c. (29)). The reasons for the absence of

emetic effects are unknown. However, *Suncus murinus* does possess opioid receptors, as morphine and loperamide can antagonize drug-induced emesis via naloxone-sensitive sites (29). The role of ∂ and κ receptors in mediating emesis has not yet been addressed in this species. However, it may be important to note that after a 15 minute pretreatment with naloxone, 2 mg/kg s.c., loperamide, 2 mg/kg s.c., can be revealed to have an emetic potential (29) or alternatively, loperamide may be revealing the emetic potential of naloxone. Possible explanations for this are discussed below.

PECULIARITIES CONCERNING OPIOID DRUG-INDUCED EMESIS

The interpretation of results from the cat regarding the unmasking of the emetic potential of 'normally non-emetic' opioid drugs (11) requires caution but has implications that may be important for the clinical use of these agents. Firstly, it was apparent that the dose of naloxone (administered intravenously or intraperitoneally) that was used to reveal the emetic potential of the normally 'non-emetic' opioid drugs, was unable to prevent morphine-induced emesis. The experiments also demonstrate that naloxone was only capable of preventing morphine-induced emesis and the emesis induced by the normally 'non-emetic' opioid drugs (as revealed by systemic naloxone) when administered at a high dose of 1 mg/cat i.c.v. But the situation becomes increasingly complicated, as the same dose of naloxone administered intracerebroventricularly to prevent emesis was also emetic *per se*. Emesis induced by naloxone was not prevented by area-postrema lesion.

It is unclear why naloxone should reveal the potential of fentanyl (and the other normally 'non-emetic' opioid drugs) to induce emesis if fentanyl acts at the same class of μ opioid receptor to mediate both effects. Certainly, when fentanyl was administered to induce emesis, the concentration of naloxone at the receptor sites within the area postrema would not be considered sufficiently high to prevent morphine-induced emesis (or the emesis to the normally 'non-emetic' opioid drugs). It is possible, however, that the dose of naloxone was high enough to antagonize or reduce a normal inhibitory tone within the 'vomiting centre' (as revealed by the emetic potential of naloxone in area postrema-ablated animals). It is also possible that different opioid receptor subtypes mediate emesis and anti-emesis, or that the endogenous opioid tone within the 'vomiting centre' is normally very low, or simply that the opioids mediate their effects via other non-opioid receptors.

Unmasking the emetic effects of normally 'non-emetic' opioids with naloxone in the cat experiments may be important clinically in understanding the mechanisms that operate during the observed increase in the incidence of PONV with the use of normally 'non-emetic' opioid drugs such as fentanyl (3). Such a rationale could be taken into account in the design of future studies investigating the underlying mechanisms of PONV.

However, it must also be questioned as to why naloxone fails to unmask the emetic potential of normally 'non-emetic' opioid drugs in the ferret and the dog (see

above for references). One simple explanation is that the conditions underwhich the cat, dog and ferret experiments were performed are different (e.g. fentanyl was administered intracerebroventricularly in the cat, intravenously in the dog and subcutaneously in the ferret; naloxone was used as a 1-hour pretreatment in the cat at a dose of 1 mg/kg i.p. or i.v., in the dog naloxone was administered as a 15-minute intravenous pretreatment at 0.07 mg/kg, and the ferret received 1 mg/kg s.c. as a 15-minute pretreatment). Difference in experimental design may be critical to an interpretation of the results in which non-specified peripheral effects of opioids may influence central emetic mechanisms (e.g. bilateral vagotomy can attenuate the analgesic effects of morphine (30)).

It must not be discounted that other species differences may also exist. For example, morphine 6-glucuronide, an active metabolite of morphine, is a potent analgesic drug that does not induce emesis in humans (31), but is a potent emetic in the ferret. The emesis in the ferret can be prevented by naloxone, 0.5 mg/kg s.c., in a predictable manner (23). Certainly, the evidence presented so far indicates that different species have different sensitivities for moderately selective agonists to induce emesis, and it should not be overlooked that there may also be differences in the metabolism of opioid drugs in different species (23).

Finally, it is worth considering that naloxone has been observed to precipitate emesis in humans when used to reverse the respiratory depressant effects of morphine (32). Is it possible that during these studies, naloxone preferentially antagonized an endogenous inhibitory opioid tone at the 'vomiting centre' to reveal emesis to the residual morphine acting at the area postrema. However, our understanding of opioid mechanisms in the control of emesis is further complicated by attempts to explain the mechanism of nausea and vomiting occurring as a result of abstinence to opioids in opioid-addicted animals and humans, or by precipitation of the abstinence syndrome using naloxone or partial agonists (33–35). Such a dissection of the emetic mechanisms is complicated, but may indicate that chronic opioid use may down-regulate a normal endogenous inhibitory opioid-tone at the 'vomiting centre'.

ANTI-EMETIC ACTION OF OPIOID DRUGS

Naloxone is well known to induce emesis when administered intracerebroventricularly at a high dose, even in the absence of the area postrema (11, 15, 36, 37). Naloxone can also induce emesis when given intravenously at doses ranging from 25 to 56 mg/kg i.v. in a number of species (37, 38); in this respect, the cat appears to be the most sensitive, with emesis sometimes occurring at doses ranging from 3 to 5 mg/kg i.v. (39).

An ability of an antagonist to induce emesis, albeit at high dose, may be indicative of a normal opioid inhibitory tone within the 'vomiting centre'. Thus, treatment with non-emetic doses of naloxone can also potentiate the emetic effects of other emetogenic drugs, regardless of their mechanism of action. Naloxone (at

sub-emetic doses) has been demonstrated to potentiate apomorphine-, copper sulphate-, cisplatin-, and zacopride-induced emesis in the ferret (19, 40), and importantly, increases the nausea to apomorphine in humans (41), and increases nausea and vomiting experienced by cancer patients receiving chemotherapy (42).

Conversely, agonists should be expected to prevent emesis. Thus morphine can prevent emesis induced by morphine itself, acetyl strophathidine dibutyryl cAMP, veratrum, staphylococcus enterotoxin, nicotine, apomorphine, copper sulphate, and cyclophosphamide (10, 11, 14, 23, 29, 43). The ability of morphine to prevent apomorphine-induced emesis is also shared by fentanyl, levophanol and methadone (11, 14). Loperamide is also capable of causing a self-blockade of its own emesis (27).

To the authors' knowledge, only one short documented clinical study exists to report the anti-emetic properties of an opioid agonist to control emesis in humans. FK 33–824 was administered to patients receiving chemotherapy and was reported to have some beneficial action in reducing emesis (although the data were inconclusive (44)). However, it has also been observed that alfentanil can prevent PONV (45), but most studies indicate that opioids increase the incidence of PONV (3). Moreover, it is well known that morphine is capable of causing a self-blockade of its own emesis in humans (33).

Fentanyl (at doses as low as 20 μg/kg s.c.) is also able to prevent emesis induced by morphine, apomorphine, nicotine, copper sulphate and cisplatin, in the dog, ferret and *Suncus murinus* via naloxone-sensitive sites (14, 19, 20). Even more convincing evidence is obtained from the use of sufentanil, which can prevent apomorphine-induced emesis in the dog at low doses (0.28 μg/kg i.v. (46)). The anti-emetic effects of fentanyl in the ferret and *Suncus murinus* are not reversed by quaternary derivatives of naloxone, suggesting that the anti-emetic effects are mediated in brain areas within the blood-brain barrier (Rudd, unpublished data).

There appears to be overwhelming evidence to indicate a role for μ opioid receptors in the modulation of the anti-emetic effects of opioid drugs in all the animal species studied so far. These opioid (μ) receptor agonists seem capable of preventing emesis, regardless of the mechanism involved to induce the response (i.e. via the area postrema and/or vagal pathways). Less convincing evidence is obtained from the use of selective μ opioid receptor agonists in humans, where fentanyl can increase the incidence of PONV (see above). This is puzzling and may indicate that the μ-receptor activation is not capable of preventing all forms of emesis. The operative procedures or surgical adjuncts may stimulate or sensitize pathways that are not directly replicated in the animal studies. However, it is also possible that the surgery or anaesthesia may interfere with the normal endogenous opioid tone within or at the 'vomiting centre', to reveal emesis by mechanisms discussed above. Conversely, the μ receptor may simply not be involved in modulating broad-spectrum anti-emetic control.

The role of other opioid-receptor subtypes in the control of emesis has not been studied so intensively. Nevertheless, it appears that the ∂ receptor may not be involved in anti-emetic mechanisms as DALA and met-enkephalin fail to prevent

apomorphine-induced emesis, at least in the dog (14). It is not certain, however, if these compounds penetrate deeper medullary structures, and further investigations in the area are required.

The role of κ receptors is also unresolved. In the dog, the non-selective κ opioid receptor agonists bremazocine and EKC prevent apomorphine-induced emesis (14), but tifluadom fails to prevent morphine-induced emesis (9). Further, in the ferret, U 69593 is emetic at low doses. There are no studies to indicate an anti-emetic effect of κ opioid receptor agonists in humans and detailed studies using selective compounds are required to understand the role of the κ opioid receptor in anti-emetic mechanisms.

PHARMACOLOGY OF OPIOID-INDUCED EMESIS

It is difficult to assess the role of anti-emetic drugs to prevent opioid-induced emesis in humans, particularly as most of the reports concerning the use of opioids are related to PONV. It is known that PONV is probably the result of a number of causative factors and that successful anti-emetic agents that reduce PONV do not necessarily need to function by interfering with mechanisms activated by the use of opioid drugs (47). Thus, our knowledge is based mainly on the use of animal models, which may not be directly applicable to the problems occurring in man. Most of the experiments conducted in animals have tended to investigate the anti-emetic mechanism of action of drugs under conditions in which emesis is driven to the theoretical maximum (ED_{100}).

Early clinical trials have indicated, however, that metoclopramide, proclorperazine and droperidol are effective in controlling morphine-induced emesis (see above), and more recently, ondansetron has been demonstrated to reduce the nausea and vomiting induced by morphine (49). Such observations implicate a role for dopamine and 5-hydroxytryptamine (5-HT) in emesis induced by opioids in humans.

In the ferret, however, the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) fails to affect loperamide-induced emesis (27), and $5\text{-}HT_3$ receptor antagonists are similarly ineffective at reasonable doses (23, 24, 27). However, droperidol (3 mg/kg i.v., but not 1 mg/kg (24)) can antagonize emesis induced by morphine, but domperidone fails to antagonize the response in the ferret (27).

Resinferatoxin, a potent capsaicin analogue, and CP 99,994, a neurokinin$_1$ (NK_1) receptor antagonist, are respectively capable of preventing loperamide- and morphine-induced emesis (49, 50), suggesting a role for substance P in the modulation of the response. However, NK_1 receptor antagonists appear to act as broad-spectrum anti-emetics, and the role of substance P may be common to the vomiting reflex and not specific for opioid-induced emesis.

Other drugs that antagonize morphine-induced emesis include 8-hydroxy-2-(di-n-propylamine) tetralin (8-OH-DPAT) (51), which may interfere with the input of

information through the 'vomiting centre' (see Chapter 23) and histamine receptor antagonists (36). As opioids are known to be more emetic in ambulatory patients (3), it is possible that histamine receptor antagonists interfere with an action of morphine at the vestibular system, as antihistamine compounds are effective in treating motion-induced emesis (52).

MECHANISMS INVOLVED IN THE ANTI-EMETIC ACTION OF OPIOIDS

An appreciation of the emetic and anti-emetic properties of opioid receptor ligands requires an understanding of the role of endogenous opioids within the brainstem. Endogenous opioids and receptor sites are present in the nucleus tractus solitarius, and stimulation of the vagus nerve can result in analgesia (30). Furthermore, μ opioid receptors are located presynaptically on vagal afferent fibres (53) and may act to interfere with the integration of information between emetic detector sites before they input to the 'vomiting centre'.

It is tempting to speculate that μ opioid receptors may be involved in the control of substance P release, which acts at NK_1 receptors to cause emesis. This premise seems logical as both μ opioid and NK_1 receptors modulate broad-spectrum anti-emetic effects and the hypothesis is testable. However, if NK_1 receptor antagonists fail to prevent naloxone-induced emesis, then the order of interaction in the inhibitory/facilitatory cascade may be reversed. It is possible, however, that μ opioid receptor agonists do not control substance P release (or some other important neurotransmitter substance) in the 'vomiting centre', and this may explain the absence of effect of fentanyl to prevent PONV. Finally, although broad-spectrum anti-emetic agents can influence vomiting, it is not yet known whether such drugs are capable of reducing nausea. A failure to control nausea may limit their clinical value.

CONCLUSION

Opioid receptor agonists appear to be capable of inducing emesis in a number of different species by activation of μ, ∂ or κ receptors. The severity of the emesis may be linked to the relative efficacy of the agonist for a particular opioid receptor (depending on the species studied), but also seems to be related to the lipophilicity of the agonist. The use of molecular biology to reveal a further sub-division of the existing opioid receptor family is challenging to the design of selective agonists and antagonists (54). The discovery of more subtypes of opioid receptors provides an exciting environment to search for opioid receptor drugs that do not induce emesis and are free from other unwanted side-effects, such as respiratory depression. The anti-emetic mechanisms of action of opioid drugs appears to be mediated by μ opioid receptors in many species but the role of κ receptors awaits confirmation.

With the advances in the discovery of new opioid receptor subtypes (54), it may eventually be possible to dissociate pharmacologically the anti-emetic properties of opioid receptor agonists from other unwanted effects, to produce a relatively clean broad-spectrum anti-emetic drug, and to identify the detailed pharmacology of opioid receptors involved in emesis and anti-emesis.

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Chapter 23

5-HT_{1A} receptor agonists as anti-emetics

J. B. Lucot

INTRODUCTION

The anti-emetic drugs most commonly used in clinical practice are antagonists that block transmission along one of the predominant afferent pathways. In the presence of such antagonists, stimuli that activate other pathways, including redundant ones, remain effective in triggering the reflex. The treatment of vomiting elicited by stimuli of unknown origin, or with the ability to activate multiple pathways, would be simplified by the availability of drugs with a broad spectrum of anti-emetic effects. It has been suggested that such a drug would act on a neurotransmitter process that is either crucial for the integration of emetic afferents or is involved in the emetic efferent signal (1). These possibilities may underlie the broad-spectrum anti-emetic effects of drugs that reduce the transmission of substance P (see Chapter 25). The 5-HT_{1A} receptor agonists are broad-spectrum anti-emetics in several species. This action appears to result from an active, physiologically relevant mechanism that is inhibitory to vomiting.

THE ANTI-EMETIC EFFECTS OF 5-HT_{1A} RECEPTOR AGONISTS

Species and stimuli

The cat

The prototypical 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamine) tetralin (8-OH-DPAT) (DPAT) has been tested for its ability to block the vomiting elicited by five stimuli, several of which act through different predominant pathways (2, 3). Provocative motion requires vestibular input but not an intact area postrema; xylazine, an α_2 adrenoceptor agonist, requires the area postrema; intragastric administration of copper sulphate requires peripheral afferents; cisplatin requires peripheral afferents and may use other pathways; RU 24969 stimulates various 5-HT_1 receptor subtypes and acts at an unknown location.

DPAT was given subcutaneously over the range of 10 to 640 μg of the base per kg. It blocked vomiting elicited by each stimulus, but at different doses. The rank

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order of sensitivity to inhibition by DPAT was motion > xylazine > RU 24969 > cisplatin. The dose of 640 μg/kg was tested for its ability to inhibit vomiting elicited by 10 mg of copper sulphate in 20 ml of water given by nasogastric tube. The vomiting was completely inhibited, though the relative sensitivity of oral copper sulphate-induced vomiting to inhibition by DPAT remains to be determined (Table 1).

Suncus murinus

Two 5-HT_{1A} receptor agonists, DPAT and SUN 8399, were tested for their ability to prevent vomiting elicited by five stimuli (4). The stimuli were provocative motion, nicotine (which acts on the area postrema, and possibly on peripheral afferents), veratrine (which acts through the nodose ganglia), oral copper sulphate and cisplatin. DPAT was given subcutaneously over the dose range of 11.3 to 3600 μg/kg. The rank order of sensitivity to inhibition by DPAT was motion > nicotine > veratrine > copper sulphate > cisplatin. Interestingly, the rank order of sensitivity for SUN 8399, given subcutaneously from 125 to 4000 μg/kg, was different, with nicotine > motion > veratrine > cisplatin > copper sulphate. The reason for the different rank order between the two drugs is not clear.

The ferret

DPAT given subcutaneously from 250 to 750 μg/kg was tested for its ability to prevent vomiting elicited by apomorphine and morphine, which are thought to act through the area postrema, as well as oral copper sulphate and cisplatin (5). The rank order of sensitivity to inhibition by DPAT was apomorphine > morphine > oral copper sulphate > cisplatin.

The pigeon

DPAT, given intramuscularly from 20 to 640 μg/kg, and LY 228729, given intramuscularly from 10 to 160 μg/kg, were tested for their ability to suppress vomiting elicited by a putative σ agonist, ditolyguanidine (DTG), whose site of action is unknown. The highest dose of DPAT was also tested for its ability to inhibit

Table 1. Effects of DPAT on vomiting elicited by oral administration of copper sulphate.

Pretreatment	Number vomiting/number tested	Average latency (SE)
Saline	5/5	23.02 (3.45)
DPAT 640 μg/kg	0/5	–
Saline	5/5	34.68 (3.40)

Subcutaneous injections were made 15 minutes prior to intragastric administration of 10 mg of copper sulphate in 20 ml H_2O. Latency refers to the time elapsed to the first retch.

vomiting elicited by 0.5 mg/kg of tropisetron and by conditioning (6). Conditioned vomiting was considered to occur when the latency to the first retch on three consecutive tests with DTG was less than 4 minutes, compared with the average latency of 18–22 minutes. The 5-HT_{1A} receptor agonists inhibited vomiting elicited by DTG, conditioning and tropisetron.

Hence, the efficacy of 5-HT_{1A} receptor agonists as anti-emetics has been demonstrated in four species. The emetic stimuli that are responsive to the anti-emetic effect include provocative motion, drugs that act on the area postrema, drugs that act on peripheral afferents, conditioned vomiting and cisplatin. DPAT has been tested in four species and the sensitivity of different stimuli to inhibition is consistently motion > drugs acting on the area postrema > oral copper sulphate > cisplatin.

Specificity of action at 5-HT_{1A} receptors

A potential difficulty in the interpretation of pharmacological studies is that any given drug may have actions at unidentified receptors. To evaluate this possibility, the rank order of potencies of a series of drugs from different chemical families may be compared with the predicted rank order of potencies based on receptor affinities and intrinsic activities. The intrinsic activity is the ability of a drug to activate the receptor after binding. Partial agonists such as BMY 7378, buspirone and ipsapirone have intrinsic activities of only about 0.1, 0.23 and 0.28, respectively, whereas full agonists such as DPAT, flesinoxan and LY 228729 have intrinsic activities of 1.0 (7).

Such an analysis for cats leads to the prediction that the rank order of potencies should be LY 228729 > flesinoxan > DPAT > BMY 7378 > buspirone. This is identical to the relative potencies obtained for these drugs in the suppression of motion sickness (3, 8). A similar evaluation of the published data of drugs suppressing motion sickness in *Suncus murinus* (4) again produces both a predicted and obtained rank order of potencies of DPAT > ipsapirone > buspirone. It is highly unlikely that these drugs would have the identical rank order of potencies at an unidentified receptor as at the 5-HT_{1A} receptor.

Tolerance

Some responses to 5-HT_{1A} receptor agonists rapidly develop tolerance. Should the anti-emetic effect be one, it would limit the usefulness of these agonists as anti-emetics in long-lasting syndromes such as cyclic vomiting, cisplatin-induced vomiting, and prolonged exposure to abnormal motion environments. To address this possibility, five pigeons were administered 640 μg/kg of DPAT on 16 consecutive days (6). On days 5, 9, 12, and 16, they were challenged with an emetic dose of DTG 15 minutes after receiving DPAT. Only one pigeon vomited after DTG, and only on day 9.

A less rigorous schedule of DPAT was evaluated in cats. The dose of 40 μg/kg of DPAT salt (30 μg/kg of the base) was administered on 3 consecutive days, with vehicle injection followed by provocative motion on Day 4 (9). DPAT produced no

Table 2. Lack of tolerance to DPAT suppression of motion sickness in cats.

Saline	3 days before test	Test on Day 4	Saline
12/21	DPAT	Saline – 17/21	16/21
16/21	nothing	DPAT – 4/2	12/21
17/21	DPAT	DPAT – 0/2	19/21

Data are numbers of animals that vomited/total number tested. DPAT was given in a dose of 40 µg/kg salt (30 µg/kg of the base). DPAT or nothing was given on 3 consecutive days, with a motion test following differing injections on Day 4. Two weeks elapsed between all tests in the first column and the start of the procedure in the second column, and between the last two columns. Data in the first two rows previously published (9).

residual effect on motion sickness, a result consistent with its short duration of action (Table 2). This dose without treatment on previous days protected all but four cats from motion sickness. This dose was then administered on 4 consecutive days, with provocative motion initiated 15 minutes after DPAT on Day 4. The anti-emetic effect was not decreased by the prior drug exposure, rather it appeared to be enhanced. The behaviour changes produced by higher doses of DPAT became evident on the third and fourth consecutive days of DPAT administration.

The results from both studies failed to find evidence for the development of tolerance to the anti-emetic effect. However, dose schedules that more accurately reflect clinical use patterns remain to be done, to ensure that tolerance does not develop under actual use conditions.

Mode of action

Two approaches were used to test the hypothesis that the 5-HT_{1A} receptor agonists inhibit vomiting by stimulating the autoreceptors that suppress 5-HT neuronal firing, thus removing a link in the chain of neural steps leading to vomiting (3). One approach was to mimic this presynaptic action by other means, such as depleting the stores of 5-HT or nonspecifically blocking 5-HT receptors. Depletion of 5-HT in the cat with the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) did not decrease the incidence of motion sickness, rather it decreased the latency to the first retch (3). Similarly, depletion of 5-HT in the ferret with reserpine or fenfluramine did not suppress vomiting elicited by oral copper sulphate (5). Metergoline was used to block 5-HT_1 and 5-HT_2 receptors before testing with either provocative motion (3) or xylazine, two emetic stimuli that are not blocked by 5-HT_3 receptor antagonists. The incidence of vomiting was not reduced in either test (Table 3).

The other approach was based on a comparison of anti-emetic doses of 5-HT_{1A} receptor agonists with those doses that suppress firing of 5-HT neurones in awake cats. Two doses of DPAT that were higher than the dose that completely suppressed 5-HT firing continued to produce dose-dependent inhibition of vomiting elicited by xylazine and RU24969 (3).

Table 3. Lack of effect of metergoline on vomiting elicited by subcutaneous administration of 0.66 mg/kg of xylazine base.

	Dose of metergoline (mg/kg)				
	0	0.3	1.0	3.0	0
Number vomiting/ number tested	10/10	10/10	10/10	10/10	10/10
Average latency	5.02	4.60	4.74	6.75	6.66
Standard error	1.67	0.46	0.49	1.49	2.04

Metergoline was administered subcutaneously 3 hours before xylazine to permit maximum absorption and penetration into the CNS.

In each approach, the conclusion is that the anti-emetic effects result from a postsynaptic site of action. The 5-HT_{1A} receptor agonists are not acting on autoreceptors to suppress 5-HT neurones serving as a link in the neural sequence that underlies emetic mechanisms. Rather, they are mimicking neurotransmission in a neural pathway that is inhibitory to vomiting. The identity of the physiological stimulus that activates this inhibitory pathway is unknown. One speculation is that it may be activated by a threat that is more immediate than that posed by the ingestion of a toxin. The only evidence lies in anecdotes of Second World War naval and air gunners who rapidly overcame motion sickness to defend against a sudden enemy attack (10).

There is little evidence that can be used to deduce the neural mechanism underlying the anti-emetic effect. One possibility is that stimulation of 5-HT_{1A} receptors provides an inhibitory signal to the sum of stimulatory signals from emetic stimuli. Alternatively, stimulation of 5-HT_{1A} receptors could enhance a neural pattern of firing that excludes that which mediates vomiting.

The latter possibility is indirectly supported by results from studies of coughing, which involves a different respiratory pattern generator from vomiting (11). Cough and vomiting both share the description of being a response to stimuli that are relayed through the nucleus tractus solitarius. Both are also inhibited by stimulation of postsynaptic 5-HT_{1A} receptors (12). In conscious whole animals, 5-HT_{1A} agonists induce an increase in respiration. By augmenting the respiratory pattern generator, the agonists may make it more difficult to switch to the pattern generators that subserve coughing and vomiting. Should this speculation prove accurate, it would imply that the nucleus tractus solitarius serves as a switch that selects among different respiratory pattern generators rather than as a simple relay.

CONCLUSION

Stimulation of 5-HT_{1A} receptors by different agonists effectively suppresses vomiting elicited by a wide variety of stimuli in four species. Tolerance to the anti-

emetic effect does not develop rapidly, so that this mechanism may be clinically useful. The receptors appear to be located postsynaptically, which indicates that they are part of a physiologically relevant anti-emetic pathway that remains to be characterized.

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Chapter 24

Muscarinic cholinergic mechanisms in emesis

J. R. R. Stott

INTRODUCTION

For the sea-voyaging public of the 19th century, seasickness was clearly a problem. There is more than a hint of desperation about the many advocated medicaments, amusingly reviewed by Reason and Brand in their book, *Motion Sickness* (1). They include opium, strychnine, creosote, amyl nitrite, ipecacuanha, hydrocyanic acid and, first mentioned in 1869, tincture of belladonna in combination with chloroform. Although there were further reports in the medical literature of the value of belladonna, and its purified derivatives, atropine and hyoscine, it was not until the Second World War that more systematic research was undertaken to confirm the efficacy of hyoscine (scopolamine), and to a lesser extent that of atropine, in motion-induced vomiting.

THEORETICAL BASIS OF MOTION-INDUCED EMESIS

In parallel with developments in treatment, ideas were evolving about the aetiological mechanism of this seemingly purposeless emetic response to travelling in ships, aircraft or road vehicles, riding on fairground amusements, or even partaking in such activities from the static comfort of a cinema seat. The recognition as early as 1881 of the necessity for a functioning vestibular system, the emergence of sensory conflict rather than excessive motion stimulation as the provocative stimulus, and more recently, the ideas of Treismann (2) setting motion sickness into a wider emetic context, have been key steps towards the understanding of motion-induced vomiting.

The current perspective is that vertebrates possess within the central nervous system a mechanism that monitors the neural signals that represent orientation information. This information is derived principally through the visual perception of motion, and from signals derived from the semi-circular canal and otolithic components of the vestibular system. There is an expectation that at all times, but particularly during locomotion, there will be a visual frame of reference that remains earth-fixed, and a vestibular-sensed gravitational framework that remains constant in direction and intensity. In the interests of postural regulation the organism has a need to ensure that signals derived from these multiple sources remain mutually consistent with this representation of the external world. Experiments on the

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interaction of these sensory inputs indicate that the brain is extremely sensitive to any discrepancies. It appears that the process of evolution has come to use this system as a detector for an emetic mechanism, based on the premise that failure to conform to expected rules of integration of motion sensory information implies a malfunction within the central nervous system that might be the result of ingested neurotoxins.

MECHANISM OF ACTION OF MUSCARINIC ANTAGONISTS

Evidence that the motion-sickness emetic mechanism is distinct from that involving serotonin comes from a double-blind, placebo-controlled study that compared the 5-HT_3 receptor antagonist, ondansetron, 8 mg, with hyoscine, 0.6 mg, in the prophylaxis of motion-induced nausea (3). A cross-coupled motion challenge in 24 subjects at 90 minutes post-dose was significantly better tolerated after hyoscine, whereas the response to ondansetron showed no hint of a benefit over placebo. Though anticholinergic agents are not the only group of drugs that act on the motion-sickness emetic mechanism, drugs that are effective appear to have an action within the CNS, by contrast with the anti-emetic action of the $5HT_3$ receptor antagonists, which may be largely peripheral. The site of the anti-emetic action of muscarinic cholinergic antagonists, or indeed of any anti-motion sickness drug is unknown. The sensory-conflict model of motion sickness allows some predictions. If a drug were to modify the sensory input, say, by reducing some component of vestibular neural activity, it might be more likely to cause nausea and vomiting by upsetting the expected sensory balance between visual, semi-circular canal and otolithic inputs. Possible brain locations for the sensory comparator function are the flocculo-nodular and uvular regions of the cerebellum, and the lateral vestibular nucleus. It might be predicted that anti-emetic drugs act beyond this point in the neural pathway, but not before it.

SUBTYPES OF MUSCARINIC RECEPTOR

The classification of acetylcholine receptors into the two classes, muscarinic and nicotinic, first put forward by Sir Henry Dale in 1914, has in recent years been elaborated through pharmacological and molecular biological methods. Muscarinic and nicotinic receptors belong to two separate gene superfamilies. The genes for at least five muscarinic receptor subtypes (m_1–m_5), have now been identified by cloning techniques (4). The development of the selective antagonists, pirenzepine, AF-DX 116, hexahydro-siladifenidol and tropicamide, has resulted in the identification of four receptors (M_1–M_4), corresponding to the cloned receptors, m_1–m_4. The muscarinic receptor protein is thought to consist of seven trans-membrane helical segments of about 24 amino acids, linked by intra- and

extracellular loops. This structural pattern is common to all G protein-coupled receptors such as the adrenergic and dopamine receptors, and the visual pigment rhodopsin. By contrast, the nicotinic receptor has five subunits, each with only four trans-membrane segments, a feature shared by the 5-HT_3 receptor.

Both atropine and hyoscine have broadly equal affinities for all muscarinic receptor subtypes. The efficacy of hyoscine in the prophylaxis of motion-induced vomiting is limited by the side-effects of drowsiness, dry mouth and blurring of vision if the oral dose is increased beyond about 0.9 mg. With the availability of selective antimuscarinic drugs, the possibility exists of targetting the emetic pathway without incurring the side-effects produced by blockade of other receptors. The muscarinic receptor antagonist idaverine, which, relative to hyoscine, has a 72-fold binding affinity for the M_2 receptor and a 12-fold affinity at M_1, showed no prophylactic benefit in motion sickness in cats (5). We have investigated the anti-emetic properties of a selective M_3 receptor antagonist, zamifenecin, in human volunteers (6). This compound has an affinity at the M_3 receptor that is equivalent to that of atropine, whereas at the M_1 and M_2 receptors its affinity is less by a factor of 50 and 100, respectively. Studies using cloned muscarinic receptors have also shown zamifenecin to have a high affinity for the m_3 receptor, but also for the m_5 receptor. The drug was administered to 18 subjects in a dose of 20 mg, and compared with placebo and hyoscine, 0.6 mg in a double-blind replicated factorial design. Motion sickness tolerance was assessed at 90 minutes after drug administration using cross-coupled stimulation, in which subjects made sequences of head movements on a turntable whose rotational velocity was incremented at 2°/second every 30 seconds. Subjects continued to make sequences of head movements until an endpoint of moderate nausea. By comparison with placebo, hyoscine increased cross-coupled tolerance by 5.65 (SE ± 1.55) sequences and zamifenacin by 4.97 (SE ± 1.55) sequences ($p < 0.01$ for both comparisons, ANOVA and Newman-Keuls test). The difference between zamifenecin and hyoscine was not significant. This finding suggests that the receptor subtype involved may be the M_3 receptor, but the affinity of this drug for the m_5 cloned receptor cannot be overlooked, nor has the M_4 receptor been positively excluded.

OTHER STIMULI TO THE MOTION-INDUCED EMETIC MECHANISM

The vomiting associated with an acute episode of vertigo, such as occurs in acute labyrinthitis and Menière's disease, or vomiting that follows middle- or inner-ear surgery are probably the result of conflicting motion signals emanating from the vestibular system and therefore may be treated as a form of motion sickness. However, if discordant motion stimuli are to be regarded as an unfortunate and inappropriate trigger to this cholinergic emetic mechanism, the question arises, what toxic insults to the brain are appropriate stimuli to elicit vomiting by this mechanism?

Opiate-induced emesis and motion

There is some evidence that the nausea and vomiting that follows opiate administration occurs through the motion-sickness emetic mechanism. It has long been known that if subjects are allowed to move about after being given morphine, the incidence of vomiting is increased. In one study, 162 ambulatory subjects were given morphine orally, intravenously or subcutaneously in doses ranging from 8 mg s.c. to 30 mg orally (7). No nausea or vomiting was reported in a placebo group of 26 subjects. Of 15 mobile subjects given 15 mg s.c., 84% complained of dizziness, 40% experienced nausea, and 16% vomited. This was compared with a group of 68 recumbent patients who were given the same dose of subcutaneous morphine, of whom only 12% reported nausea in a 1 hour pre-operative period, and none vomited. This study found no reduction in the incidence of nausea in 16 ambulatory subjects who received 0.6 mg atropine together with 15 mg morphine subcutaneously. However, the increase in postoperative nausea and vomiting that is associated with the use of morphine premedication has been found to be reduced by the simultaneous administration of atropine, 0.6 mg i.m., and still more effectively by hyoscine, 0.4 mg i.m. (8). It has also been shown that no anti-emetic benefit is conferred by glycopyrrolate, a muscarinic anticholinergic that does not cross the blood-brain barrier (9). Furthermore, it is reported that opiate-induced nausea and vomiting shows little or no response to $5HT_3$ antagonists.

MUSCARINIC ANTAGONISTS AND THE PROCESS OF HABITUATION

One feature of motion-induced nausea and vomiting is that, with repeated exposure to provocative motion, the individual becomes increasingly resistant to its nauseogenic effects. This process of habituation is affected by hyoscine. In a study using repeated measures (10), subjects received for 3 successive days either 1 mg hyoscine, 0.6 mg hyoscine with 10 mg amphetamine, 10 mg amphetamine, or placebo. Their tolerance to cross-coupled motion was measured on each day at 1 hour post-dose, and again on Day 4 in the absence of medication. The hyoscine with amphetamine and the hyoscine conditions were associated with an apparently faster rate of habituation. However, on Day 4 when no drug had been given, the tolerance of these two groups was found to be significantly less than for the placebo condition. A similar observation was made during a 7-day sea trial (11) in which the hyoscine patch, which delivers a loading dose of 200 μg hyoscine followed by 20 μg/hour for up to 72 hours, was used for the first 3 days of the trial, and compared with a placebo group who wore an inactive patch. For the first 2 days, the incidence of seasickness was significantly less in the hyoscine patch group, though not on the third day. However, on removing the patch, the incidence of seasickness on Day 4 was significantly greater in those who had used the hyoscine patch.

CONCLUSIONS

The process of evolution has preserved this CNS-based, muscarinic cholinergic emetic mechanism, despite the seemingly more immediate, and potentially more effective, gut-based 5-HT_3 system. The fact that the muscarinic emetic mechanism has come to light through the study of motion sickness has given some insight into the way in which it might function. Once the relevant receptors are known, it may be possible to develop more effective drugs to suppress this emetic pathway. Such drugs are likely to be of wider therapeutic use than simply for motion sick travellers. In terms of increased understanding, the techniques of molecular biology and immunocytochemistry offer the prospect of localizing relevant receptor subtypes and complementing what is already known from neurophysiological studies of visual-vestibular interaction. The next 10 years should see some exciting developments.

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Chapter 25

The tachykinins and emesis: towards complete control?

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INTRODUCTION

The discovery of 5-HT_3 receptor antagonists has led to a dramatic improvement in the control of emetic side-effects during cancer chemotherapy. However, although 5-HT_3 receptor antagonists are effective against the acute emetic response induced by radiation and cytotoxics, they fail to block the emetic responses to most centrally acting emetic stimuli (opioid and dopaminergic agonists), certain peripherally acting emetic stimuli (copper sulphate) and motion (1). More importantly, 5-HT_3 receptor antagonists fail to reduce the delayed nausea and emesis associated with cytostatic therapies in man.

The utility of the 5-HT_3 receptor antagonists in the treatment of acute cytotoxic-induced illness has stimulated research into the mechanisms and pharmacology of emesis. Numerous neurotransmitters have been implicated, however substance P, by virtue of its localization within the area postrema and nucleus tractus solitarius, and its ability to induce emesis when administered to the vasculature (2), was considered likely to have a role in emetic responses. As substance P is the preferred ligand for the NK_1 receptor, development of non-peptidal NK_1 receptor antagonists by Pfizer Inc., led us to evaluate these compounds as anti-emetics. One of these compounds, CP-99,994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylperidine) has proven particularly useful by virtue of its potency and selectivity for the NK_1 receptor, and the availability of a 2R,3R enantiomer, which is inactive as a NK_1 receptor antagonist (3). The utility of CP-99,994 for investigating the possible involvement of NK_1 receptors in emesis has also been recognized by others (4–6).

ANTI-EMETIC ACTIVITY OF CP-99,994

The NK_1 receptor antagonist CP-99,994 has proven to be an extremely broad-spectrum anti-emetic inhibiting responses to cytotoxics, central, peripheral and mixed emetic agonists, and motion. In the ferret, CP-99,994 (0.1–1 mg/kg s.c.), but not its NK_1 receptor inactive enantiomer, CP-100,263 (1 mg/kg, s.c.), inhibits both the acute (Figure 1) and delayed (Figure 2) emetic response to the cytotoxic drug cisplatin (10 mg/kg i.p.) (7; Nagahisa *et al.*, unpublished observations). CP-93,009, a racemic mixture that includes CP-99,994, has also been shown to inhibit cyclophosphamide- (200 mg/kg i.p.) and radiation-induced (200 CGy) emesis in this

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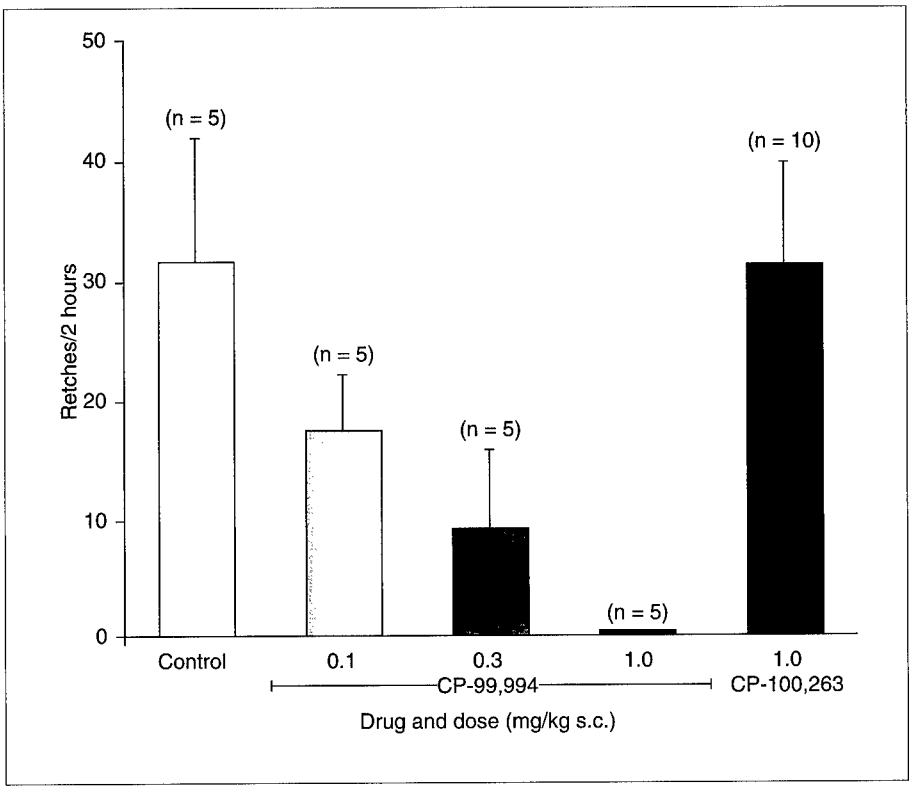

Figure 1. Effect of CP-99,994 and CP-100,263 on the acute retching response to cisplatin, 10 mg/kg i.p., in ferrets. CP-99,994 and CP-100,263 were dosed at 1 mg/kg s.c., 45 minutes after cisplatin.

species (4). In addition, CP-99,994 (0.1–1 mg/kg s.c.), but not CP-100,263 (1 mg/kg s.c.), provides dose-related inhibition of the emetic response to the gastric irritant copper sulphate (12 mg/kg p.o.), the opioid receptor agonist loperamide (250 μg/kg s.c.), and the dopamine D_2 receptor agonist apomorphine (200 μg/kg s.c.) (7, 6). We have also shown that CP-99,994 (40 μg/kg + 5 μg/kg/minute i.v., or 3 mg/kg s.c.) provides protection against copper sulphate (6 mg/kg p.o.) and apomorphine (10 μg/kg i.v.) emesis in dogs (7), and copper sulphate-(40 mg/kg p.o.) induced emesis in *Suncus murinus* (Nagahisa, unpublished observations). In addition, CP-99,994 inhibited the emetic response to motion induced by a lab shaker in *Suncus murinus* (10 mg/kg s.c.) (Figure 3, Nagahisa *et al.*, unpublished observation), and induced by a ferris wheel using cats (0.3 mg/kg s.c.) (Lucot *et al.*, unpublished observation). Finally, CP-99,994 potently (1 mg /kg i.v.), and reversibly (response wanes after 90 minutes) inhibited the retching response to abdominal vagal afferent electrical stimulation (25V, 40 Hz, 0.5 mseconds) in urethane-anaesthetized ferrets (7).

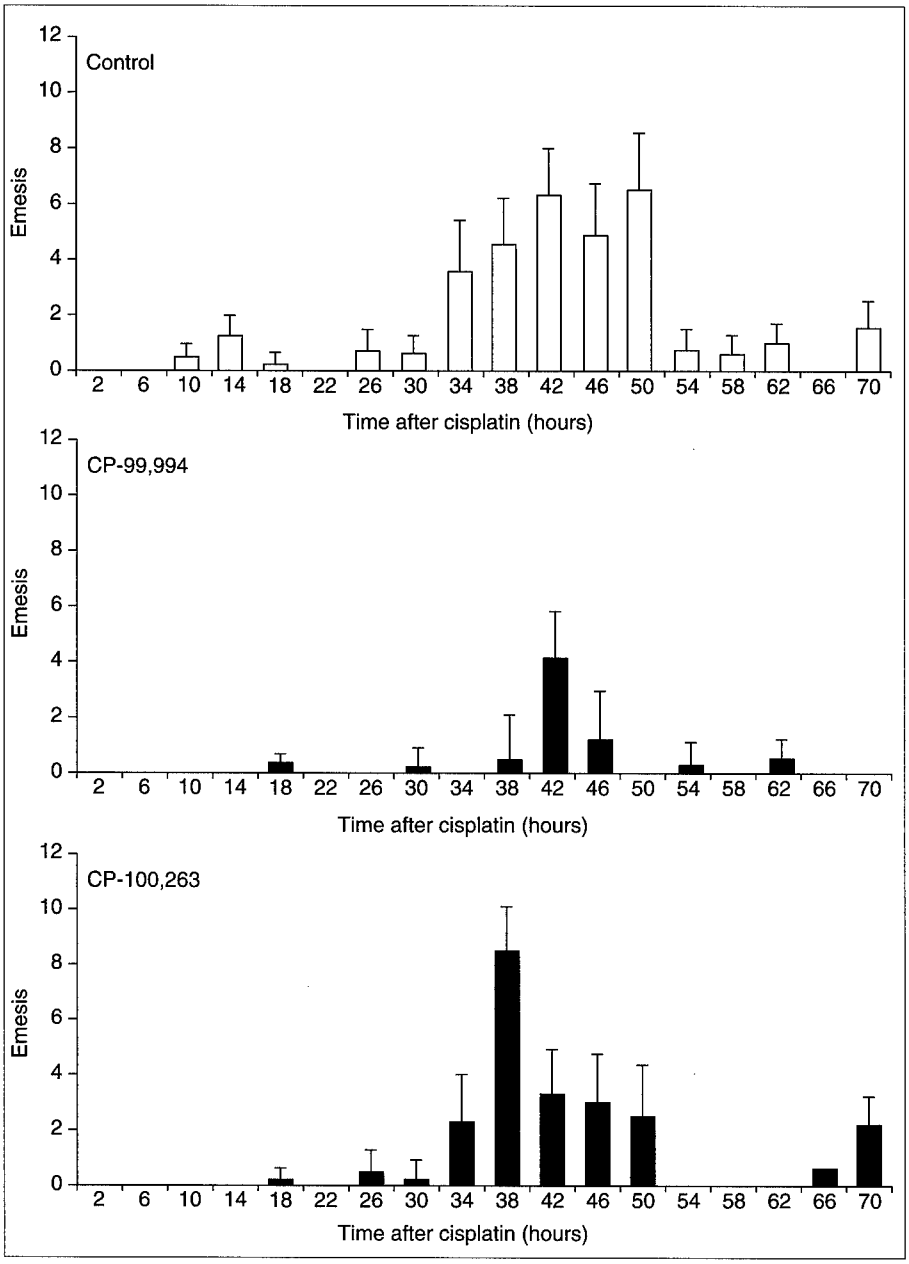

Figure 2. Effect of CP-99,994 and CP-100,263 on the delayed emetic response to cisplatin, 5 mg/kg i.p., in ferrets. CP-99,994 and CP-100,263 were dosed at 10 mg/kg s.c., 30 minutes before and 8, 24, 32, 48 and 56 hours after cisplatin. Emesis is quantified as frequency of vomiting or retching.

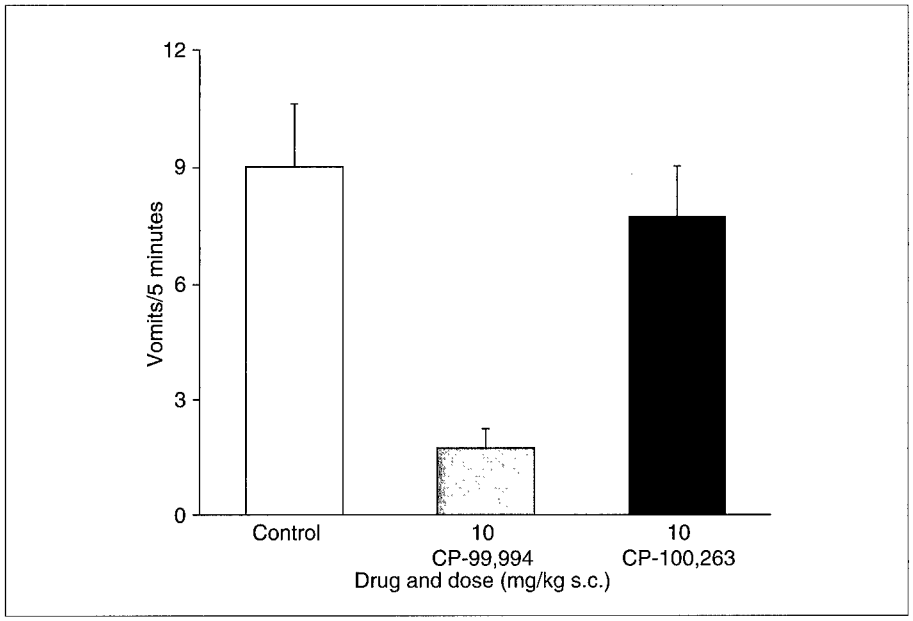

Figure 3. Effect of 10 mg/kg of CP-99,994 and CP-100,263, dosed 60 minutes before challenge, on motion-induced vomiting in *Suncus murinus*. The vomiting response to motion produced by a laboratory shaker was quantified during a 5-minute observation period.

In contrast to its inactivity in other emetic models, CP-100,263 provided dose-related inhibition of ipecacuanha-induced emesis in the ferret, although with only a fraction (0.1) of the potency expressed by CP-99,994. The anti-emetic activity of CP-100,263 cannot be attributed to NK_1 receptor antagonism, as the compound lot was examined and found to be inactive (IC_{50} > 10 μM) in a [^{125}I] Bolton Hunter Substance P IM9 cell receptor binding assay (7). Although this observation suggests that CP-100,263 expresses anti-emetic activity, its limited spectrum suggests that the mechanism of action is distinct from that of CP-99,994. The pharmacological properties of CP-100,263 merit further investigation.

SITE OF ACTION OF CP-99,994

Although the anti-emetic site of action of CP-99,994 has not been completely elucidated, the available evidence strongly supports a site within the brainstem, potentially within the nucleus tractus solitarius. First, CP-99,994 effectively blocks the emetic response to chemicals known to exert their effect centrally, including loperamide, apomorphine and morphine. In addition, CP-99,994 blocks motion-induced emesis and abdominal vagal afferent stimulation-induced retching, reflexes

both known to be mediated within the CNS. Second, potent NK_1 receptor antagonists that are not distributed to the brain but that have demonstrated potent peripheral NK_1 receptor antagonist activity *in vivo* are inactive against emetic responses (Watson, unpublished observation (8)). Also highlighting the requirement for brain penetration, the NK_1 antagonist GR 82334 failed to inhibit emetic responses to cisplatin in ferrets when administered intravenously, but was quite effective when administered into the brainstem (9). Third, small volumes of peptidal NK_1 receptor antagonists (Sendide and Spantide II) administered to the cisterna magna (i.c.v.) inhibited intraduodenal hypertonic saline-induced retching and vomiting in halothane-anaesthetized ferrets (10). Fourthly, the distribution of CP-99,994 displaceable [^{3}H]substance P binding within the ferret brainstem suggests a site of action within the NTS (7). Although the available evidence suggests that CP-99,994 has a central site of action, this does not exclude an action at a peripheral site (e.g. vagal afferents responding to mucosally released substance P) for some stimuli (e.g. copper sulphate).

SELECTIVITY OF *IN VIVO* FUNCTIONAL EFFECTS OF CP-99,994

Despite the broad distribution of immunoreactive substance P and specific [^{3}H]substance P binding sites within the nucleus tractus solitarius, including areas thought to be involved with homeostatic cardiovascular and respiratory reflexes, the *in vivo* effects of CP-99,994 are quite circumscribed (7). At doses known to inhibit emetic responses in the ferret, CP-99,994 had no effect on baseline blood pressure, heart rate, respiratory rate and tidal volume. In addition, it had no effect on a number of reflexes including: the reflex bradycardia; stimulation of respiratory rate and depth; and the subsequent reflex increase in blood pressure following intravenous administration of 2-methyl 5-HT in urethane-anaesthetized ferrets (von Bezold-Jarisch reflex); the swallowing and gag reflexes evoked by pharyngeal stimulation; and transient apnoea during electrical stimulation of the cervical vagus (modified Hering Breuer reflex) in the ferret.

SUMMARY

As reviewed, the available data strongly implicate substance P and NK_1 receptors in the central neural network that mediates emetic responses. The ability of CP-99,994 to modulate emetic responses to central and peripheral chemical, cytotoxic, and labyrinthine stimuli is unprecedented among other receptor antagonists. Its ability to block the delayed emetic response to the cytotoxic drug cisplatin is unprecedented in any class of agent. With the exception of anticipatory or emotionally induced emesis, against which it has yet to be evaluated, CP-99,994 has been shown to block emesis regardless of cause. And yet, CP-99,994 has been shown to have no effect on laryngeal, respiratory or cardiovascular reflexes that are known to be mediated

through the same region of the brainstem responsible for emetic reflexes. This remarkable specificity clearly enhances the therapeutic potential of NK_1 receptor antagonists as anti-emetics. Although the anti-emetic effects of CP-99,994 have been demonstrated in *Suncus murinus*, ferrets, dogs and cats, anti-emetic effects in humans would require conservation of the central role of substance P in the emetic reflex. If this is the case, then NK_1 receptor antagonists could have greater potential than 5-HT_3 receptor antagonists in the treatment of nausea and vomiting.

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Serotonin and the control of emesis: a decade of progress?

Abstracts presented at

Exeter College, Oxford

27–29 March 1995

Index to abstracts

P1: Augmentation of cisplatin-induced emesis by repetitive treatment of cisplatin in *Suncus murinus*

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The effects of repetitive administration of cisplatin, pyrogallol (a generator of free radicals), or 2-methyl-5-HT (a selective 5-HT_3 receptor agonist), on cisplatin-induced emesis were studied in *Suncus murinus*, the house musk shrew. Cisplatin (10 mg/kg/day), pyrogallol (65 mg/kg/day), 2-methyl-5-HT (5 mg/kg/day) or vehicle was injected intraperitoneally for 3 days, and then the emetic response to 20 mg/kg cisplatin was tested on the fourth day. In the cisplatin-treated group, emetic responses were augmented compared with the vehicle-treated group. The latency to the first vomiting was significantly shorter. Emetic responses to 10 mg/kg cisplatin itself were also enhanced during 3 days' pretreatment. However, the enhancement was not observed when the animals were treated with pyrogallol or 2-methyl-5-HT. These drugs caused emesis during the pretreatment. The cisplatin-treated animals responded more vigorously to 2-methyl-5-HT injected on the fourth day, but not to pyrogallol. The amount of 5-HT and its metabolite, 5-HIAA, in the intestine were measured after repetitive treatment with cisplatin. However, these values of the cisplatin-treated group were not significantly different.

It was not necessary to inject cisplatin for 3 days to sensitize animals. The augmentation of 20 mg/kg cisplatin-induced emesis on the fourth day was observed when 10 mg/kg cisplatin had been injected once (first day) or twice (first and second day). However, when 20 mg/kg cisplatin-induced emesis was tested on the eighth day after a single injection of 10 mg/kg cisplatin on the first day, the augmentation was not significant.

Pretreatment with tropisetron (200 μg/kg/day), a selective 5-HT_3 receptor antagonist, during the repetitive administration of cisplatin, blocked the augmentation of cisplatin-induced emesis on the fourth day.

These results suggest that repetitive administration of cisplatin specifically and reversibly exaggerates the subsequent cisplatin-induced emesis, probably through the augmentation of 5-HT receptor-mediated response in the terminals of vagal afferents or in the enterochromaffin cells in the intestinal mucosa.

P2: Ondansetron compared with granisetron in the control of cisplatin-induced emesis

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There has been speculation that minor pharmacological differences between 5-HT_3 receptor antagonists indicates that there is clinical superiority for one of the agents over another. However, these suggested pharmacological differences are small and largely hypothetical. A number of relatively small open studies have compared 5-HT_3 receptor antagonists in patients receiving a variety of cytotoxic regimens. These studies have reported that these agents are equivalent, or that there are small differences. The efficacy of anti-emetic agents in clinical trials is dependent on the emetogenicity of the chemotherapy and patient factors that predispose patients to emesis. Clinical studies designed to compare 5-HT_3 receptor antagonists must be well balanced with regard to these factors. We have conducted, therefore, the first large randomized, double-blind, parallel-group study to compare a single intravenous dose of ondansetron (ond) (8 or 32 mg) with granisetron (gran) (3 mg) in the control of acute cisplatin-induced emesis. A total of 496 patients were randomized to receive one of the three anti-emetic treatments prior to cisplatin chemotherapy ($\geqslant$ 50 mg/m^2). The treatment groups were well balanced with respect to age, gender, body surface area, alcohol use, cisplatin dose (median dose 78 mg/m^2), and concomitant chemotherapy. All three treatments were highly effective. There were no significant differences in the efficacy (Table) or tolerability of the three anti-emetic regimens. Headache was the most reported drug-related adverse event for all three treatment groups and occurred in 9% of all patients.

Treatment group	Complete control of emesis (%)	Complete or major control of emesis (%)	Nausea: none or mild (%)
Ond 8 mg (n = 165)	59	76	71
Ond 32 mg (n = 162)	51	74	69
Gran 3 mg (n = 169)	56	78	73

Ond, ondansetron; Gran, granisetron.

Previous studies comparing 8 and 32 mg of ondansetron have either found that there is no difference between these two doses or that the 32 mg dose is significantly superior. There may therefore be some patients in whom the 32 mg dose of ondansetron provides optimal control of emesis. The results from this study illustrate that comparisons of 5-HT_3 receptor antagonists from open studies in which patients receive a variety of chemotherapeutic agents with differing emetogenic potential should be interpreted with caution. Moreover, this study shows that suggested clinical differences between ondansetron and granisetron, which have been based on pharmacological hypotheses, do not exist.

P3: The role of serotonin as a mediator of emesis induced by inner ear dysfunction, pregnancy or cisplatin therapy

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Emesis is a uniform answer to diverse stimuli, and can be induced by drugs, motion and other conditions like pregnancy. The mechanisms of these different types of emesis are only partially understood. To evaluate the impact of changes in serotonin metabolism on the pathophysiology of different types of emesis: pregnancy-associated emesis, emesis induced by inner ear dysfunction, and cisplatin-induced emesis. Urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of serotonin was measured in 13 women with pregnancy-induced emesis, 12 patients who suffered from vertigo, nausea and vomiting following inner ear surgery or other inner ear dysfunctions, 27 patients with cisplatin-induced emesis, and a control group of 21 women. Urine collection was performed at 2, 4 and 8 hour intervals over 3 consecutive days and corresponding nausea and vomiting was recorded in a diary. 5-HIAA was measured with a fluorescence-polarization-immunoassay (Abbott) and corrected for varying urine concentrations (5-HIAA/creatinine ratio).

The 5-HIAA/creatinine ratio varied between 3.5 and 6.8 in the control group. No common circadian rhythm could be detected. The 12 patients with emesis associated with inner ear dysfunction showed a similar 5-HIAA excretion pattern, and no correlation between intensity of nausea or vomiting and changes in 5-HIAA excretion could be detected. The 5-HIAA/creatinine ratios of the patients with pregnancy-induced emesis showed no statistical difference compared to the other two groups. Again, no correlation between emetic episodes and changes in 5-HIAA excretion could be observed. In patients receiving cisplatin, the 5-HIAA excretion increased significantly within the 12 hours following cisplatin administration and returned to baseline levels after 24 hours. The peak 5-HIAA/creatinine ratio ranged from 15 to 25 with a mean of 17.8. There was a parallel increase in 5-HIAA excretion and numbers of emetic episodes in the first 12 hours following chemotherapy. A significant number of emetic episodes occurred later than 24 hours after chemotherapy, but 5-HIAA excretion remained at the baseline level. Our results suggest that serotonin is involved only in one of four investigated types of emesis: cisplatin-induced acute emesis. Cisplatin-induced delayed emesis, pregnancy-associated emesis, and emesis caused by inner ear dysfunction are not associated with elevated levels of 5-HIAA excretion. Probably, the serotonin pathway represents only one (of many different?) afferent mechanisms capable of initiating the emetic cascade. Chemotherapy-induced delayed emesis as well as pregnancy-associated nausea and vomiting are still a therapeutic dilemma, and further studies are warranted to investigate the mechanisms underlying them.

P4: An investigation of the role of 5-HT_4 receptors in emesis in the ferret

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In ferrets, the gastroprokinetic agent zacopride has been shown to induce emesis (1), which can be blocked by a high dose (1 mg/kg), but not by a low dose (0.1 mg/kg) of the 5-HT_3 receptor antagonist, tropisetron (2). Similarly, high doses of tropisetron, but not of the 5-HT_3 receptor antagonists, granisetron or ondansetron, were found to antagonize copper sulphate-induced emesis in the ferret. Bhandari and Andrews (2) speculated that these actions of tropisetron were caused by blockade at 5-HT_4 receptors.

We have investigated the effects of a potent and highly selective 5-HT_4 receptor antagonist, GR125487, [1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl] methyl 5-fluoro-2-methoxy-1H-indole-3-carboxylate (3) in emesis induced by R,S-zacopride (0.4 mg/kg p.o.), cisplatin (200 mg/m^2 i.p.) or copper sulphate (40 mg/kg p.o.) in ferrets. For comparison, granisetron and ondansetron were tested in cisplatin- and copper sulphate-induced emesis, and granisetron in zacopride-induced emesis. To investigate possible synergy, ondansetron combined with GR125487 was tested against cisplatin-induced emesis.

Neither granisetron (1 mg/kg s.c.) nor GR125487 (1 mg/kg s.c.) antagonized zacopride-induced emesis (mean number of retches ± SEM in 2 hours post emetogen treatment: control 23 ± 9.0, granisetron 26 ± 17.9, GR125487 16 ± 7.6). GR125487 (1 mg/kg s.c.) was also ineffective in antagonizing emesis induced by cisplatin (control 104 ± 22.3, treated 115 ± 40.9 retches) or copper sulphate (control 95 ± 37.5, treated 85 ± 8.5 retches). Both granisetron (0.1 mg/kg s.c.) and ondansetron (0.1 mg/kg s.c.) inhibited cisplatin-induced emesis (granisetron: control 102 ± 13.9, treated 11 ± 10.5; ondansetron: control 107 ± 30.0, treated 12 ± 12.0 retches). However, neither granisetron (1 mg/kg s.c.) nor ondansetron (1 mg/kg s.c.) inhibited emesis induced by copper sulphate (granisetron: control 95 ± 37.5, treated 80 ± 18.6; ondansetron: control 166 ± 49.6, treated 117 ± 45.9 retches). Ondansetron (0.1 mg/kg s.c.) and GR125487 (1 mg/kg s.c.) given in combination were no more effective than ondansetron alone against cisplatin-induced emesis (control 107 ± 30.0, treated 5 ± 2.9 retches). In conclusion, 5-HT_4 receptors are not involved in emesis induced by any of the agents used in ferrets.

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2 Bhandari P *et al. Eur J Pharmacol* 1991; **204:** 273–280.
3 Gale JD *et al. J Gastroint Mot* 1993; **5:** 192.

P5: Attenuation of SSRI-induced GI disturbance by the 5-HT$_3$ receptor antagonist, ondansetron

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Selective serotonin re-uptake inhibitors (SSRIs) are increasingly being used to treat depression and anxiety disorders and, in some cases, unpleasant side-effects can lead to poor compliance or discontinuation of treatment before any benefit can be gained. The most commonly reported side-effects of the SSRI class of drugs are those of nausea and gastrointestinal upset. A possible mechanism for this is thought to be stimulation of 5-HT receptors either on abdominal vagal afferents or in the area postrema (1).

In a double-blind placebo-controlled study in 10 healthy volunteers, we investigated the possibility that fluvoxamine-induced GI disturbance could be reduced by administration of the 5-HT$_3$ receptor antagonist ondansetron. After baseline measurements, subjects were given 100 mg fluvoxamine orally at 09.30, together with either 8 mg ondansetron or placebo and, because of the short half-life of ondansetron, a second dose was given at 12.30. At 10.00 and then hourly until 16.00, subjective ratings of nausea and GI symptoms were made using visual analogue scales. Each condition was 1 week apart.

Individual peak nausea ratings were significantly higher on placebo compared with the ondansetron treatment ($p < 0.05$; Wilcoxon). On the ondansetron day, 40% of subjects rated their nausea > 10 mm at any time during testing, as opposed to 80% on the placebo day ($p = 0.08$; Fisher's exact test). For total GI symptoms experienced, ANOVA showed a significant treatment–time interaction ($F = 2.39$; df = 1,8; $p = 0.02$). They were significantly lower in the ondansetron than in the placebo group at 14.00 ($p = 0.02$). For total symptoms experienced the ANOVA for treatment–time interaction ($F = 2.44$; df = 1,8; $p = 0.02$) was also significant at 13.00 ($p = 0.01$) and 14.00 ($p < 0.005$), the ondansetron group having the lower symptom scores.

The way in which SSRIs cause nausea and other gastrointestinal symptoms has not been fully elucidated. They may prevent re-uptake (2) of 5-HT released by enterochromaffin cells into the intestinal mucosa, leading to stimulation of local vagal afferents and thereby nausea. This study suggests that the GI disturbance induced by fluvoxamine may be mediated by 5-HT and attenuated by the 5-HT$_3$ receptor antagonist ondansetron.

1 Kilpatrick GJ *et al. Eur J Pharmacol* 1989; **159:** 157–164.
2 Legay C *et al. Neurochem Int* 1983; **5:** 721–727.

P6: The effect of alteration of 5-HT synthesis on cisplatin (10 mg/kg)-induced emesis in the ferret during a 24-hour observation period

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The ferret has been extensively utilized as a model to investigate the mechanism(s) of action of cisplatin (CP) to induce emesis. The studies have typically used an ED_{100} dose of CP (i.e. 10 mg/kg) to induce emesis within a 4-hour observation period. The emesis occurring during this period is sensitive to treatment with 5-HT_3 receptor antagonists and is prevented by 5-HT synthesis inhibition using parachorophenylalanine (PCPA; 1). However, using extended observation periods, we have recently described a component of CP- (10 mg/kg) induced emesis that is partially resistant to treatment with the 5-HT_3 receptor antagonist alosetron (2). The present study investigates the role of 5-HT in emesis induced by CP (10 mg/kg) in the ferret during a 24-hour observation period.

Ferrets (0.75–1.3 kg) were pretreated with PCPA (100 and 200 mg/kg i.p.) or vehicle (1% tween 80, 2 ml/kg; Veh) for 4 days. CP (10 mg/kg) or saline (0.9% w/v, 5 ml/kg; Sal) was administered intraperitoneally 4 hours after the last dose of PCPA or Veh. At 24 hours, the ferrets were killed and a urine sample and tissue samples from the dorsal vagal complex (DVC) and mid-ileal mucosa were removed and analysed for 5-HT and 5-HIAA content by HPLC-ECD; creatinine was measured colorimetrically (Table). Emesis was recorded as previously described (2).

CP non-significantly increased 5-HT and 5-HIAA levels in the DVC by 126 and 22%, respectively ($p > 0.05$). The levels of 5-HT and 5-HIAA were reduced by up to 96 and 99%, respectively, by the combined PCPA/CP treatment ($p > 0.05$). CP non-significantly reduced 5-HT and 5-HIAA levels in the mucosa by 69 and 73%; such reductions achieving significance when combined with PCPA 200 ($p < 0.05$). 5-HT was not detected in the urine but CP significantly increased urinary

Treatment	DVC		Mucosa		Urine
	5-HT	5-HIAA	5-HT	5-HIAA	5-HIAA
Veh:Sal	333.0 ± 34.5	160.0 ± 27.7	122.1 ± 62.1	227.8 ± 61.3	0.9 ± 0.1
Veh:CP	752.1 ± 232.0	195.0 ± 18.7	37.9 ± 15.5	171.6 ± 37.1	2.7 ± 0.1*
PCPA 100:CP	31.0 ± 5.5†	18.7 ± 3.0*†	13.9 ± 3.3	80.6 ± 31.5	1.0 ± 0.5†
PCPA 200:CP	30.1 ± 5.7†	1.7 ± 2.6*†	6.4 ± 3.1	12.6 ± 3.6†	0.1 ± 0.0†

Tissue 5-HT and 5-HIAA levels are in pg/mg wet weight; urinary 5-HIAA levels are μg/mg creatinine (*$p < 0.05$, significantly different to Veh:Sal; †$p < 0.05$, significantly different from Veh:CP).

5-HIAA levels (μg/mg creatinine) by 200%; the increase was significantly antagonized by combination with PCPA (Table).

Animals treated with Veh:Sal failed to exhibit emesis but Veh:CP induced 114.5 ± 25.9 retches + vomits (RV) during the total 24-hour observation period; 68.2 ± 6.6% of the response occurred within the first 8 hours. PCPA 100 and 200 mg/kg significantly reduced the % of RV that occurred during the first 8-hour period by 70 and 55%, respectively ($p < 0.05$). However, during the remaining 16-hour observation period, the RV in PCPA 100- and 200 mg/kg-treated animals compared with Veh:CP-treated animals was increased by 207 and 92%, respectively ($p > 0.05$).

In conclusion, the data may indicate that a reduced 5-HT function may be effective in antagonizing the first 8-hour phase of RV induced by cisplatin, 10 mg/kg, but is revealing of a later phase of emesis that is independent of 5-HT synthesis.

1 Barnes JM *et al. Neuropharmacology* 1988; **27:** 783–789.
2 Rudd JA *et al. Neuropharmacology* 1994; **33:** 1607–1608.

P7: Temporal changes of the gastrointestinal 5-HT levels following cisplatin treatment in the piglet

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Vomiting in cancer chemotherapy is classified as acute or delayed when occurring during the first day following cytotoxic administration or thereafter, respectively. The introduction of selective 5-HT_3 receptor antagonists represents a major advance in the prevention of vomiting induced by antineoplastic therapy. Recent clinical data suggest that, although 5-HT_3 receptor antagonists are effective in the control of the delayed-phase of cisplatin-induced vomiting, their efficacy seems somewhat lower than that achieved during the acute phase. Some authors have suggested that this may result from the possibility that a non-serotonin-mediated mechanism is more prevalent during the delayed phase.

The present work attempted to clarify the role of the 5-HT in delayed cisplatin-induced emesis. Using the model of delayed emesis we have developed recently in the piglet, we measured the gastrointestinal tissue levels of 5-HT during:
1) the emetic peak of the acute phase (+ 1.5 hours);
2) the transition period between the acute and delayed phases (+ 16 hours);
3) the onset of the delayed phase (+ 21 hours);
4) 60 hours after cisplatin dosing (+ 60 hours).

Piglets were sacrificed by exsanguination under deep sodium thiopental anaesthesia. Tissue was immediately dissected out from the stomach, the duodenum, the ileum and the colon. Mucosae were peeled off, frozen and stored at –80° C until analysis. On the day of analysis, samples were homogenized with an ultrasonic distruptor and centrifugated. Serotonin in the supernatant was separated using a reversed-phase column and measured by HPLC with electrochemical detection. The protein concentrations in the homogenates were determined by a modified Lowry procedure. The results are expressed as ng of 5-HT/mg of protein.

The 5-HT concentrations of control piglets (n = 6) were 7 ± 1.6; 26 ± 1.7; 5 ± 0.7 and 8 ± 1.2 (n = 8) in the gastric, duodenal, ileal an colonic mucosae, respectively. The 5-HT level in the duodenal mucosa significantly increased (91 ± 25, n = 4) 1.5 hours after cisplatin dosing, decreased at +16 hours (43 ± 3.05, n = 3), returned to the control level at +21 hours (23 ± 2.9, n = 2) and then presented a new increase at + 60 hours (53 ± 7.4, n = 3). There were no significant changes in the 5-HT levels in the ileal (n = 3) and colonic (n = 4) mucosae at + 1.5 hours (4 ± 0.8; 7 ± 0.4). Increased levels of 5-HT were found at + 16 hours and + 60 hours in the ileum (8 ± 0.7; 12 ± 2.6, n = 3), but only at + 60 hours in the colon (18 ± 4.5, n = 3). There were no significant changes in the level of 5-HT in the gastric mucosa (n = 3–6) during either the acute or the delayed phase. Pretreatment with granisetron (7 mg/kg) reversed the cisplatin-induced increase in 5-HT level in the duodenal mucosa (44 ± 11, n = 3; $p < 0.05$ *versus* cisplatin alone) observed at +1.5 hours in cisplatin-treated piglets.

These preliminary results demonstrate that cisplatin increased the duodenal 5-HT level during both acute and delayed phases, suggesting that 5-HT might play a role in the delayed phase, by a mechanism that remains to be elucidated.

P8: Contractile mechanisms of the gastrointestinal morbidity of cisplatinum

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Cisplatinum produces significant gastrointestinal morbidity (nausea, vomiting and diarrhoea) that sometimes limits therapy. We investigated the aetiology of these gastrointestinal symptoms by studying small-bowel motor activity in chronically instrumented dogs after administration of cisplatinum.

Eight small-intestinal strain gauges were implanted in eight dogs. After control recordings, cisplatinum (0.5, 1.0 or 2.0 mg/kg) was given intravenously to prehydrated dogs. Using ANOVA, there was an increase in the incidence of retrograde giant contractions (RGCs; small intestinal contractions that precede vomiting). The first RGC occurred 148 ± 26 minutes and the final 246 ± 25 minutes after the start of cisplatinum infusion.

	Control	0.5 mg/kg	1.0 mg/kg	2.0 mg/kg
RGCs/8 hours	0.33 ± 0.33	6.67 ± 2.22*	6.83 ± 1.54*	10.5 ± 1.28*
No. of vomits/8 hours	0	5.67 ± 2.26*	6.82 ± 2.12	10.5 ± 1.28*
No. of GMCs/8 hours	0.5 ± 0.22	2.67 ± 1.12	2.5 ± 0.85	7.17 ± 2.39*
No. of MMCs/8 hours	4.83 ± 0.31	3.5 ± 0.43	3.0 ± 0.26*	2.17 ± 0.31*

RGC, retrograde giant contraction; GMC, giant migrating contraction; MMC, migrating motor complex.
*$p < 0.05$ *versus* control.

Some of the RGCs were associated with more than one vomit. The frequency of emetic episodes increased dose-dependently whereas the frequency of RGCs was not different at the three doses administered. At 2 mg/kg i.v., the frequency of giant migrating contractions (GMCs; contractions that produce abdominal cramping and diarrhoea) increased significantly. The frequency of GMCs remained elevated for up to 5 days. Migrating motor complexes (MMCs; normal fasting activity of the small intestine) decreased as the dose of cisplatinum increased, but returned to normal 24 hours later.

The adverse side-effects of cisplatinum are associated with specific abnormalities of small-bowel contractile activity. The RGCs occur within hours and the GMCs persist for days. Agents that selectively block giant contractions may improve the gastrointestinal side-effects of cisplatinum and increase patient tolerance to prescribed therapy. Inhibition of those contractions may be possible with a 5-HT_3 antagonist. (Supported by DK43104; Cisplatinum provided by Bristol-Myers Squibb.)

P9: Activity of human cerebral cortex during nausea and during recovery after ondansetron, as detected using magnetic source imaging

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Considerable progress has been made in recent years in determining the input mechanisms responsible for eliciting nausea and vomiting, and how the motor act of vomiting is controlled. In contrast, the brain region(s) that produce the sensation of nausea are essentially unknown. The purpose of the present study was to use non-invasive functional neural imaging to determine if a localized cortical area is activated during nausea.

Four normal volunteers underwent magnetoencephalography (MEG) during baseline controls, nausea, and recovery. Nausea was induced by either oral ingestion of syrup of ipecac (5–10 ml) or by vestibular stimulation (induced by making head movements during yaw-axis rotation). The magnitude of nausea was scored by the subject using a 10-point scale. Functional MEG data were recorded using a 37-channel superconducting biomagnetometer (Magnes, Biomagnetic Technologies Inc., San Diego, CA). Raw magnetic tracings were analysed for the occurrence of transient bursts of focal high amplitude activity (> 400 fT). The brain location of waveforms meeting these criteria was determined using a single equivalent current dipole model. Resulting dipoles were coregistered to corresponding high resolution magnetic resonance images of the subject's brain.

Current dipoles indicative of neuronal activation were localized to a 2–3 cm diameter region of cortex in the inferior frontal gyrus. Source localizations were the same whether nausea was induced by ipecac or vestibular stimulation. More dipoles were observed during intense nausea than during milder nausea; none was observed in this region at baseline or during control sessions involving speech, finger movements or exaggerated respiratory movements. The serotonin type 3 receptor antagonist ondansetron (8 mg oral) ameliorated nausea induced by ipecac, with a resulting progressive decrease in the number of dipoles detected.

The inferior frontal gyrus appears to be important for the sensation of nausea, and thus may represent a new target for pharmaceutical intervention. In addition, the non-invasive technique of magnetic source imaging may provide a quantitative means of measuring the effects of various interventions on the subjective sensation of nausea. (Supported by grant NS20585 from the National Institute of Neurological Disorders and Stroke, USA.)

P10: Temporal relationship between autonomic nervous system (ANS) variability and chemotherapy-induced nausea

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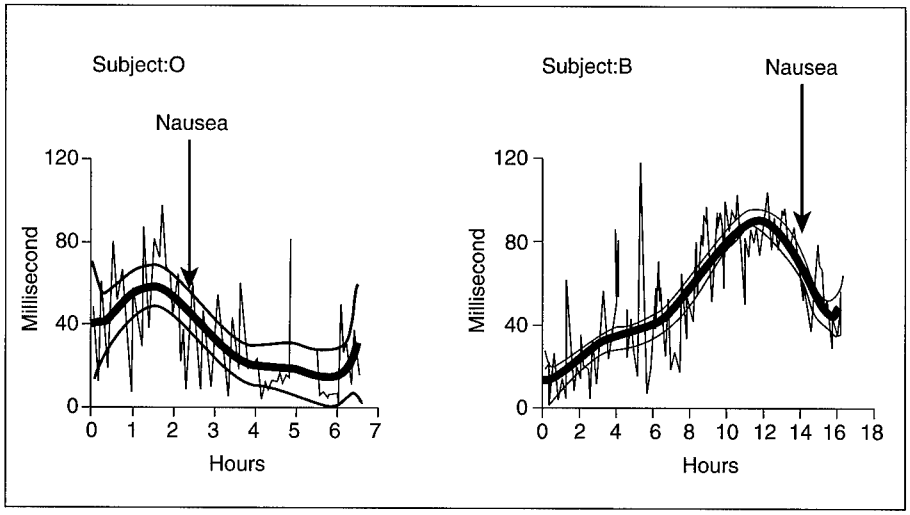
Temporal associations between ANS variability and chemotherapy-induced, patient-reported nausea were examined in 25 chemotherapy-naive ovarian cancer patients treated with either cisplatin or carboplatin. R–R intervals were assessed to < 1 ms accuracy. The standard deviation of successive R–R intervals was calculated for 5 minute intervals over 16 hours following administration of the chemotherapy, and mathematically smoothed with a seventh order polynomial. The left panel below shows typical data from a patient receiving cisplatin; the right panel below is from a patient receiving carboplatin. Peak variability was defined as the first derivative equivalent to zero. An arrow indicates the point at which the first patient reported nausea.

Consistent with clinical experience, 10 patients self-reported nausea significantly sooner following the administration of cisplatin chemotherapy than did 15 patients who received carboplatin (2.8 ± 0.7 hours *versus* 8.4 ± 2.9 hours; t = 5.8, $p < 0.5$). In 24 of the 25 cases, heart-rate variability had peaked and was in a marked decline when patients self-reported nausea. The greatest variability occurred, on average, at 1.2 (± 0.06) hours following the administration of cisplatin and 4.9 (± 3.9) hours following carboplatin (t = 2.8; $p < 0.5$). These findings support a temporal relationship between ANS variability and patient-reported nausea. ANS change was reactive to different chemotherapy drugs, and thus may be a reliable physiologic precursor of self-report nausea. (Supported by NIH-NR-01905 and NIH-RR-00044.)



P11: Upregulation of an emetic response in man?

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It has been established that syrup of ipececuanha (ipecac) will reliably induce nausea and emesis in man (1). This effect is mediated by a 5-HT_3 receptor-dependent mechanism (2). In the clinic, emesis often immediately precedes a repeat treatment with a chemotherapeutic agent (3), and patients with previous experience of emesis associated with surgical anaesthesia experience up to a three-fold greater incidence of emesis after subsequent anaesthesia (4). The mechanism of this response has yet to be characterized. In the present study, we have examined the effect of a repeat treatment with syrup of ipecac on nausea and emesis to investigate a possible mechanism for conditioned emesis. Thirty-five healthy male subjects, aged 18–45 years, participated in this double-blind, two-period, parallel-group study, in which one group (n = 18) received active ipecac on both periods (A + A), and the second group (n = 17) received placebo ipecac on the first period, and active ipecac on the second period (P + A). All subjects were given 4 mg (full anti-emetic activity) ondansetron (as the hydrochloride dihydrate) at the first period and 1 mg (partial anti-emetic activity) at the second period (i.v. infusion over 5 minutes), 30 minutes before ipecac. The number of emetic episodes, time to onset of emesis, and nausea score, based on a 0–3 (none to severe) emesis scale were recorded for up to 4 hours after giving ipecac. All procedures were approved by an independent ethical review committee.

There were no emetic episodes recorded during period one of the study, and three subjects recorded a nausea score of 1 (mild) during period one. During the second period, the proportion of subjects experiencing emesis was greater in the A + A group compared with the P + A group (61% *versus* 47%, respectively). Further, the time to onset of emesis was lowered in the A + A group compared with the P + A group (medians 167 minutes and > 240 minutes, respectively). By contrast, nausea score was significantly decreased over the 4-hour recording period in the A + A group compared with the P + A group ($p < 0.05$, Students *t*-test). Both measures of emesis were worse on repeat challenge with ipecac, compared with the group with only a single challenge. These results indicate that the emetic pathway can be upregulated over a 14-day period, and support the findings from the clinic that prior emetic experiences can worsen patients' reactions to subsequent emetic challenges. Nausea ratings were reduced over the recording period, supporting the observation that there is relief from nausea following emesis. These data support the hypothesis that although differentially regulated, nausea and emesis have a strong functional link, and may provide an insight to the mechanism behind the cycle of conditioned emesis seen in the clinic.

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P12: An investigation into the duration and site of action of ondansetron in prevention of ipecacuanha-induced emesis

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Ondansetron, as the hydrochloride dihydrate salt, is a potent anti-emetic agent, licensed for the treatment of nausea and vomiting associated with chemotherapy and radiotherapy, and surgical procedures. The duration over which ondansetron prevents nausea and emesis is an important clinical end-point and will affect a recommended dosing regimen. In a previous study, which used the syrup of ipecacuanha model of emesis in healthy male volunteers (1), the dose of ondansetron completely preventing nausea and emesis was demonstrated to be 4 mg (as an intravenous infusion over 5 minutes). Further, it has been shown that ipecacuanha-induced emesis is mediated by a 5-HT_3-dependent mechanism (2). However, the relationship between ondansetron plasma concentration and the control of emesis, and therefore the possible site of action of the drug, was not investigated. In the present study, using the syrup of ipecacuanha model of nausea and emesis, the duration of anti-emetic activity of 4 mg ondansetron was investigated and the relationship between ondansetron concentration and effect was examined.

Fifty-four healthy male volunteers, aged 18–45 years, participated in this double-blind, randomized-placebo, parallel-group study, with six treatment groups of nine subjects. Each group received 4 mg ondansetron (5 minute intravenous infusion), followed by 30 ml ipecacuanha (n = 6) or matched placebo (n = 3), which were given in an ascending order 30 minutes, 4, 6, 8, 12 and 16 hours from the start of the ondansetron infusion. The number of emetic episodes was recorded for each subject for 4 hours after dosing with ipecacuanha or placebo. All procedures were approved by an independent ethical review committee.

The number of emetic episodes recorded within each group increased with increasing time post-ondansetron. There were no emetic episodes recorded in the 30 minutes, increasing to six in the 16 hours post-ondansetron groups. There were no emetic episodes recorded in subjects receiving placebo ipecacuanha. Experimental observation has shown that the emetic challenge remains constant over the assessment period. Based on this, 48% (10/21) of the subjects were protected from emesis for up to 12.75 hours post-ondansetron. Further analysis and modelling

of the data demonstrated that there was an apparent monophasic relationship between ondansetron concentration and control emesis.

In conclusion, the present study has demonstrated that the effective anti-emetic ondansetron will prevent ipecacuanha-induced emesis in humans up to about 12 hours post-dose, and may in certain circumstances support the use of a 4 mg twice-daily treatment regimen. Further, the relationship between drug concentration and effect supports the hypothesis that ondansetron is exerting its anti-emetic effects in this model at a peripheral site of action, or at least through an effect compartment in rapid dynamic equilibrium with the plasma.

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P13: 5-HT_3 receptor antagonists inhibit enterotoxin B-induced emesis in the ferret

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5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists have been proved to be effective in a number of animal models of emesis caused by cytotoxic drugs and radiation (1). In humans, the therapeutic action of these drugs has been confirmed in clinical trials of cancer therapy induced emesis (2), and there is increasing evidence that 5-HT_3 receptor blocking agents may be effective in postoperative nausea and vomiting (3). Up until now, apart from chemotherapy and post-surgical conditions, other types of emesis have been poorly studied with regard to the efficacy of 5-HT_3 receptor antagonists. Here we present the protective action of three compounds of this class, ondansetron, itasetron and tropisetron, in a model of nausea and vomiting induced by the staphylococcal enterotoxin B in the ferret.

Male ferrets were anaesthetized with pentobarbitone (30 mg/kg i.p.) and a polyethylene cannula for drug administration was inserted into the jugular vein and exteriorized behind the neck. Animals were used 2 days after surgery. After the administration of various doses (0.3–100 μg/kg i.v.) of the highly purified enterotoxin B (Sigma), the number of emetic episodes (retching and/or vomiting) was recorded for 300 minutes. Vomits or retches occurred from 45 minutes up to 240 minutes after administration, the peak of episodes being between 60 and 120 minutes at each dose. The percentage of responders was: 25% at 0.3 μg/kg (range of emetic episodes: 0–1); 50% at 1 μg/kg (range: 0–5); 50% at 3 μg/kg (range: 0–22); 50% at 10 μg/kg (range: 0–7); 100% at 30 μg/kg (range: 5–8); 100% at 100 μg/kg (range: 1–9) (n = 4–6 per treatment group). The fully effective dose of

100 μg/kg, which induced retches or vomits in all animals, was selected for antagonist experiments. Antagonists at 5-HT_3 receptors such as ondansetron, itasetron or tropisetron (synthesized by Boehringer Ingelheim Italy), were administered immediately after enterotoxin B at the dose of 300 μg/kg i.v. (which has been reported to be fully protective against anti-cancer agents in the ferret; 4, 5); the number of emetic episodes was recorded over the following 300 minutes. Ondansetron and itasetron completely prevented the occurrence of retches and vomits (n = 5), whereas 75% of animals were protected in the tropisetron treatment group (n = 4). The present data provide evidence of efficacy of 5-HT_3 receptor antagonists in a model of emesis induced by staphyloccocal enterotoxin B. In pioneer studies, enterotoxins have been shown to be emetogenic in the monkey (6) and in the cat (7), their action being highly dependent on intact abdominal viscera. Cisplatin-induced emesis in the ferret, which is also prevented by 5-HT_3 receptor antagonists, also requires preservation of intact afferent fibres (8). It is possible, therefore, that emesis caused by staphylococcal enterotoxins, like that caused by cytotoxic agents, involves a serotonergic mechanism of stimulation of abdominal nerve fibres. Further investigations will be focussed on the mode and site of action of enterotoxin B in the induction of nausea and vomiting in the ferret. The efficacy of 5-HT_3 receptor antagonists in enterotoxin-induced emesis suggests the possibility of a new therapeutic opportunity in nausea associated with infectious diseases.

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P14: Resting vagal tone and the prediction of subsequent patient nausea from chemotherapy

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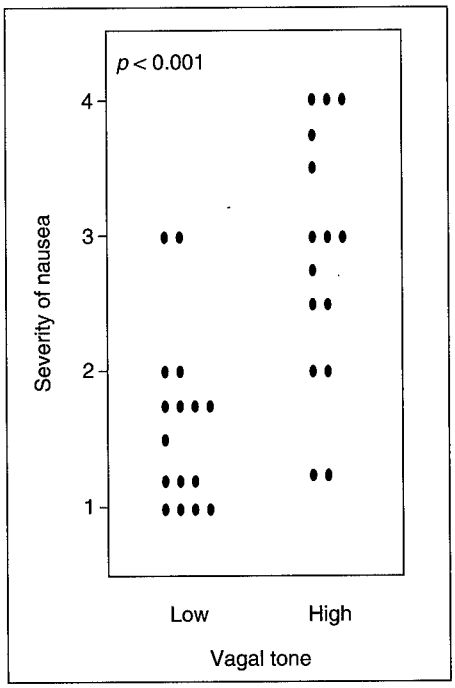
Side-effects caused by cancer chemotherapy increase patient reluctance to continue treatment and may require reduced drug dosages. These can affect patient survival. The ability to predict nausea caused by chemotherapy could lead to better control. Heart rate was monitored prior to treatment in 31 chemotherapy-naive patients subsequently treated for histologically confirmed ovarian cancer with either cisplatin or carboplatin.

ECG data were recorded with a Healthodyne monitor and 3M red dot electrodes placed on the chest at approximately the sixth intercostal space. Resting vagal tone (defined as the area under the frequency curve ± 0.06 Hz either side of respiratory sinus arrhythmia) was assessed through Fourier mathematical analysis of millisecond variations in successive R–R intervals.

The figure shows vagal tone divided into low and high subgroups through a median split *versus* subsequent patient-reported nausea severity on a four point scale of 0 = none, through 4 = severe. Patients below the median resting vagal tone had less severe subsequent nausea (1.6 ± 0.67) than patients who were above the median (2.83 ± 0.90; t(28) = 4.26; $p < 0.001$). Findings were independent of type of chemotherapy, patient age and patient report of anxiety, depression or fatigue (all $p > 0.10$). If replicated with other chemotherapy agents, these findings may help improve control of nausea/vomiting through both pharmacological and behavioural interventions by eventually making it possible to tailor individually specific anti-emetic interventions to specific patients. (Supported by NIH-NR-01905 and NIH-RR-00044.)



P15: Failure of tropisetron to inhibit copper sulphate-induced emesis in cats

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Recently it has been reported that high doses of tropisetron blocked the vagally mediated emesis induced by oral copper sulphate, suggesting that 5-HT_4 receptors are involved in emesis (1). On the other hand, 5-HT_3 receptor antagonists failed to prevent the emesis evoked by copper sulphate in ferrets (2). As tropisetron in low doses acts as a 5-HT_3 receptor antagonist and at high doses as a 5-HT_4 receptor antagonist, it was of interest to extend these studies in cats.

In an aseptic operation under pentobarbitone (35 mg/kg i.p.) anaesthesia, an infusion cannula was implanted into the left lateral cerebral ventricles of cats of either sex (2–4 kg), so that intracerebroventricular (i.c.v.) injections could be made without anaesthesia. Tropisetron was injected i.c.v. and i.v., whereas copper sulphate was administered orally. Only expulsion of gastric contents was taken as a positive response. Student's t-test was used to determine the significance between controls and various experimental groups.

The oral administration of 50 ml of the solution of copper sulphate (1%) induced emesis in 100% of cats. Emesis lasted 33.3 ± 10.5 minutes, the number of emetic episodes was 6.83 ± 1.09 and it appeared after a latent period of 8 ± 2.78 minutes. Tropisetron hydrochloride in doses from 30–300μg injected in volumes of 0.2 ml i.c.v. had no significant effect ($p > 0.05$) on the emetic responses to orally administered copper sulphate. In the next series of experiments, tropisetron in doses of 0.1 mg/kg and 1.0 mg/kg had no significant effect ($p > 0.05$) on the emetic responses to orally administered copper sulphate.

In similar experiments, tropisetron given parenterally in low doses of 0.1 mg/kg had no effect, whereas in high doses of 1.0 mg/kg it antagonized the emetic response to oral administration of copper sulphate in ferrets (1, 2). As it is assumed that tropisetron in low doses acts as a 5-HT_3 receptor antagonist and in high doses as a 5-HT_4 receptor antagonist, it is concluded that peripheral and central 5-HT_3 and/or 5-HT_4 receptor-mediated mechanisms are not involved in the emesis caused by oral copper sulphate in the cat.

1 Bhandari P *et al. Eur J Pharmacol* 1991; **204:** 273–280.
2 Costall B *et al. Neuropharmacology* 1990; **29:** 453–462.

P16: Alcohol-induced emesis in *Suncus murinus*

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It is well known that over-consumption of alcohol causes emesis. However, the mechanism whereby alcohol induces emesis has not been studied experimentally. Clinical studies have shown that patients with a history of chronic, heavy alcohol intake experience less cisplatin-induced nausea and vomiting, suggesting a correlation between alcohol- and cisplatin-induced emesis. In the present study, we investigated ethanol-induced emesis in *Suncus murinus*. Healthy adult *Suncus* of either gender were used throughout the experiments. Ethanol (4 ml/kg) caused concentration-dependent emesis with an ED_{50} value of 28.3% (i.p.) (Table). Acetaldehyde, a major metabolite of ethanol, also caused concentration-dependent emesis with ED_{50} values of 4.0% (i.p.) and 3.5% (s.c.). Shorter latency to the first vomiting was observed after acetaldehyde, suggesting that acetaldehyde rather than ethanol causes emesis.

Ethanol-induced emesis was not affected by pretreatment with 5-HT_3 receptor antagonists (zacopride and tropisetron). Abdominal surgical vagotomy also failed to prevent ethanol-induced emesis. Furthermore, intracerebroventricular injection of ethanol or acetaldehyde did not induce vomiting. These results suggest that ethanol/acetaldehyde causes emesis through the stimulation of peripheral pathway(s) distinct from the vagal afferents. There seems to be no apparent correlation between ethanol- and cisplatin-induced emesis.

Ethanol is known to increase the GABA-induced chloride current. However, phaclofen, a $GABA_B$ receptor antagonist, failed to prevent ethanol-induced emesis. 8-hydroxy-DPAT, a 5-HT_{1A} receptor agonist that prevents various types of emesis, dose-dependently blocked ethanol-induced emesis with ID_{50} value of 400 μg/kg.

	Concentration (%)	No. of *Suncus* vomiting/tested	Latency (minutes)	No. of vomiting episodes
Ethanol	20	0/5	–	–
	40	5/5	13.0 ± 1.9	11.4 ± 2.5
Acetaldehyde	4	3/9	1.0 ± 0.0	4.0 ± 2.1
	6	5/5	1.0 ± 0.3	3.2 ± 0.9

P17: Unexpected inhibition by methysergide of clonidine-induced emesis in cats

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It has been reported that alpha-adrenoceptor blocking agents (1) and atropine (2) inhibit clonidine-induced emesis in cats. However, there is evidence that clonidine might act at central 5-HT sites (3). To investigate this possibility further, an attempt was made to study the serotonergic mechanisms in emesis caused by clonidine in cats.

In an aseptic operation under pentobarbital (35–40 mg/kg i.p.) anaesthesia, an infusion cannula was implanted into the left lateral cerebral ventricle of cats of either sex (2–4 kg) so that subsequent intracerebroventricular (i.c.v.) injections could be made without anaesthesia. Only expulsion of gastric contents was taken as a positive response. Dose–response curves were constructed using linear regression according to the method of least squares. Student's *t*-test was used to test differences between treatment and control groups.

I.c.v. administration of clonidine in doses from 0.1 μg–0.3 mg induced emesis. The percentage of cats showing emesis after i.c.v. clonidine was dose-dependent ($r = 0.91$; $p < 0.01$). Clonidine-induced emesis lasted from 2–15 minutes. The number of emetic episodes ranged from 1 to 4, whereas the percentage of cats showing emesis, even with the largest doses of 0.1 mg and 0.3 mg reached 88%, but never 100%. The emetic effect was preceded and associated with licking, retching, restlessness, defaecation, micturition, ataxia and muscular weakness. In the next series of experiments, methysergide (0.01–0.3 mg) was injected intracerebroventricularly 20 minutes before the emetic challenge of clonidine (0.1 mg). Methysergide significantly ($p < 0.05$) reduced the emetic effect of clonidine. This reduction was not dose-dependent ($r = 0.57$; $p > 0.05$). In control experiments, 0.3 ml of 0.9% solution of saline injected intracerebroventricularly, as well as two repeated injections of saline in volumes of 0.2 ml and 0.3 ml at intervals of 20 minutes did not induce any visible behavioural, autonomic or motor phenomena.

In conclusion, it appears that clonidine, apart from its action at central alpha$_2$ adrenoceptors and muscarinic cholinoceptors, can affect emesis at central 5-HT receptors.

1 Hikasa Y *et al. Eur J Pharmacol* 1992; **229:** 241–251.
2 Japundžić N *et al. Eur J Pharmacol* 1990; **183:** 1929.
3 Svensson TH *et al. Brain Res* 1975; **92:** 291–306.

P18: Objective and subjective time-courses of recovery from motion sickness assessed by repeated motion challenges

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Although the process of induction of motion sickness by various provocative stimuli has been studied extensively, less effort has been directed to investigating the dynamics of recovery. The aim of this study was to determine whether the time-course of recovery of tolerance, as assessed objectively by rechallenge with motion, paralleled the subjective recovery from sickness. Subjects (n = 20) were exposed to pairs for emetogenic motion challenges in which the time interval between the end of the first and the start of the second of each pair was 15 minutes, 30 minutes, 1 hour and 2 hours. Each pair of motion challenges was performed on different days, according to a repeated-measures latin-square design. The cross-coupled motion challenge had an incrementing profile of rotational velocity from 4 to 90°/s in steps of 4°/s every 30 s, with eight head movements per 30 s of approximately 45°, and was continued to the point of moderate nausea. Data were analysed by ANOVA with specific contrasts. Objective loss of tolerance was assessed by comparing the number of sequences of head movements required to produce nausea in the first *versus* second of each pair of motion challenges.

A feature of the objective recovery was that, after an exponential recovery from 15 minutes to 1 hour, this process reversed at 2 hours when motion tolerance significantly decreased again. Subjectively, most individuals reported complete recovery by 15–30 minutes and all were recovered by 1 hour. There are few models of motion sickness recovery, reflecting the paucity of systematic experiments directed towards this area. The model of Oman postulates two summating pathways: fast neuronal (time constant, TC, 1 minute) and slow neurohumoral (TC 10 minutes) (1). Results up to 1 hour in the present experiment indicated a TC of around 7 minutes for subjective recovery, similar to the TC for the slow pathway of the Oman model, but the TC of around 20 minutes for objective recovery was much slower. Moreover the finding of reduced motion tolerance at 2 hours requires further explanation. It is possible that the longer-term dynamics of recovery are non-monotonic as a consequence of a slow damped oscillation in a neurohumoral system determining sickness susceptibility. It was concluded that there is an underlying effect of motion sickness that reduces tolerance to subsequent motion up to 2 hours later. This underlying objective effect has a much slower time-course than the subjective recovery from symptoms and appears to be non-monotonic.

1 Oman CM. *Can J Physiol Pharmacol* 1990; **68:** 294–303.

P19: Effects of racemic CP-99,994, a neurokinin NK_1 receptor antagonist, on motion-induced emesis in *Suncus murinus*

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We have previously shown that in the ferret, the neurokinin NK_1 receptor antagonist, CP-99,994 inhibits emesis induced by cisplatin, cyclophosphamide, radiation, morphine, copper sulphate and ipecacuanha (1). The present experiments have been carried out to determine the efficacy of this agent against emesis induced by motion.

Suncus murinus, the house musk shrew, is a small Asiatic insectivore that is susceptible to motion sickness, which can be antagonized by a variety of agents known also to be effective in humans (2). Emesis was induced by exposing the animals to sinusoidal acceleration using linear oscillation in a horizontal plane at 1 Hz, with an excursion of 4 cm for a period of 5 minutes. Six adult male (body weight range 53–87 g) and five adult female (33–42 g) animals were used for these experiments. The animals received a subcutaneous dose of either saline, 4 ml/kg, or racemic CP-99,994, 10 or 30 mg/kg, administered 15 minutes before motion-testing. Numbers of emetic episodes and (in the case of responders) latency to first emesis were recorded. Experiments were of a 'cross-over' design, with an interval of 13 days between treatments. All the animals received saline pre-treatment on one occasion – four on the first exposure to motion, and the remainder on the second exposure. Following administration of saline, 9 of the 11 animals vomited, and the mean number of emetic episodes was 3.7 ± 1.30. Mean latency to first emesis was 131 ± 20.3 seconds. The two animals that did not respond were excluded from further analysis. The results are shown in the Table. At the doses used, racemic CP-99,994 significantly attenuated motion-induced emesis in the house musk shrew, suggesting a role for substance P and NK_1 receptors in this response.

± CP-99,994	Control responders	Episodes	Latency (s)	Treated responders	Episodes	Latency (s)
10 mg/kg s.c.	4/4	3.8 ± 1.49	123 ± 30.1	2/4	0.8 ± 0.47*	175 ± 85.0
30 mg/kg s.c.	5/5	5.2 ± 2.46	138 ± 29.7	1/5	0.2 ± 20†	110

Values are means ± SEM, *$p = 0.04$ paired t-test, †$p = 0.06$ paired t-test

1 Bountra C *et al. Eur J Pharmacol* 1991; **249:** R3–R4.
2 Ueno S *et al. Life Sci* 1988; **43:** 413–420.

P20: An antagonist of tachykinin NK_1 receptors inhibits cytotoxic-induced plasma protein extravasation in the ferret

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Neurogenic inflammation has been demonstrated in many tissues, including the bladder and gastrointestinal and respiratory tracts (1). A cardinal feature is plasma protein extravasation (PPE). Many anti-neoplastic therapies induce adverse effects that involve inflammation. For example, haemorrhagic cystitis following cyclophosphamide (CYP) administration, or gastrointestinal damage after radiotherapy. In the present study, the effects of a tachykinin NK_1 receptor antagonist (CP-99,994) and two $5\text{-}HT_3$ receptor antagonists (granisetron and ondansetron) on PPE induced by CYP, radiation (Rx), or cisplatin (CP) were investigated in a range of tissues from the ferret.

Adult male ferrets (0.85–1.50 kg) were given a subcutaneous dose of either 0.9% saline (1 ml/kg) or an antagonist 5 minutes before administration of either CYP, CP or whole-body Rx at previously determined ED_{90-100} emetogenic doses. Anaesthesia was induced with pentobarbitone sodium (60 mg/kg i.p.) and Evans blue dye (50 mg/kg) was administered intravenously 15 minutes before the blood was flushed out with saline (time of sacrifice: see Table). The bladder, portions of the duodenum and jejunum, a kidney and lobe of lung were removed and the dye was extracted with formamide (24 hours at 60° C) and quantified by spectrophotometry (at 620 nm).

Pre-treatment with granisetron or ondansetron (1 mg/kg s.c.) had no effect on cytotoxic-induced PPE, except in the duodenum of irradiated animals (Control, 135.8 ± 16.1; granisetron, 97.5 ± 5.7; ondansetron, 75.3 ± 3.1 mg Evans blue/g tissue). However, CP-99,994 (5 mg/kg s.c.) significantly inhibited the PPE induced in the bladder after CYP, as well as in the small intestine of ferrets treated with Rx or CP (see Table for major effects of CP-99,994 on cytotoxic-induced PPE in ferrets).

These data indicate that a tachykinin NK_1 receptor antagonist attenuated some of the adverse inflammatory effects induced by cytotoxic agents in the ferret.

Cytotoxic	Time* (hours)	Tissue	PPE (µg Evans blue/g tissue)	
			Control	CP-99,994
CYP (125 mg/kg i.p.)	1	Bladder	157.8 ± 9.	63.1 ± 2.3
Rx (2 Gy)	2	Duodenum	135.8 ± 16.1	91.4 ± 9.8
		Jejunum	103.0 ± 8.4	66.8 ± 6.6
CP (10 mg/kg i.p.)	6	Jejunum	104.3 ± 11.4	69.0 ± 3.8

* Time between administration of cytotoxic agent and exsanguination.
(Values are means ± SEM, n = 3–5 per group.)

1 Maggi CA *et al. Gen Pharmacol* 1988; **19:** 1–43.

P21: The anti-emetic effects of NK_1 receptor antagonists are centrally mediated

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CP-99,994, a human neurokinin$_1$ (hNK_1) receptor antagonist, inhibits emesis induced by cisplatin in ferrets (1), a species with hNK_1-like receptors. To investigate the site of anti-emetic action of CP-99,994 we have compared the receptor binding, peripheral anti-inflammatory, brain penetration and anti-emetic activities of CP-99,994 with a novel quaternized non-peptide NK_1 receptor selective antagonist L-743,310.

The displacement of ^{125}I-Tyr^8-substance from human cloned NK_1 receptors in Chinese hamster ovary cells was used as a measure of affinity for hNK_1 receptors of CP-99,994 and L-743,310. Similar IC_{50} values of 0.5 nM and 0.1 nM, respectively were obtained, and these compounds had equivalent peripheral activity *in vivo* (ID_{50} values 7 and 2 μg/kg i.v., respectively) against plasma protein extravasation of Evans Blue dye evoked by resiniferatoxin (7 nmol/kg i.v.) in the oesophagus of anaesthetized guinea pigs.

Comparison of the brain entry of these compounds was carried out using an *in situ* brain perfusion technique in barbiturate anaesthetized rats. Compounds were dissolved in either rat plasma or phosphate buffered saline and were infused via the internal carotid artery through the brain. CP-99,994 penetrated well into brain tissue with an entry rate (K_{in}) of 0.7 ± 0.2 ml/g/minute, whereas the quaternary compound L-743,310 was poorly penetrant and had a brain entry rate of 0.002 ± 0.001 ml/g/minute, similar to the plasma marker inulin.

The effects of the hNK_1 antagonists were assessed against cisplatin-induced emesis in ferrets using a method as described previously (1). CP-99,994 (0.3–3 mg/kg i.v.) produced marked dose-dependent inhibition of retching and vomiting but L-743, 310 at doses of 3 and 10 mg/kg i.v. was without effect.

These findings suggest that the anti-emetic effects of hNK_1 receptor antagonists are centrally mediated. A central site of action for the hNK_1 receptor antagonists is also indicated by their effectiveness against such centrally acting emetogens as morphine and apomorphine.

1 Tattersall FD *et al. Eur J Pharmacol* 1993; **250:** R5–R6.

P22: Distribution of substance P (SP) binding sites in rat and ferret brainstem using quantitative autoradiography

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The distribution of tachykinin recognition sites in rat and ferret brainstem has been examined by *in vitro* autoradiography using [^{125}I] Bolton-Hunter substance P (BHSP, 0.05 nM). Segments of Wistar rat and fitch ferret brainstem approx 4 mm caudal and rostral to the obex were obtained. Coronal sections (20 μm) were cut and binding carried out according to the procedure of Helke *et al.* (1). Radiolabelled tissue sections were exposed to radiation-sensitive ^{3}H-hyperfilm for 3 days and the resulting autoradiograms were analysed using a computer-assisted image analysis system. In rat brainstem (n = 3), two distinct regions showing high BHSP specific binding (nCi/mg) were identified. These regions were within the nucleus tractus solitarius (NTS), the dorsal NTS (dNTS, 4.95) and the ventral NTS also containing the dorsal motor nucleus of the vagus (vNTS, 5.91). In addition, there was extensive binding in the inferior olive (2.90), nucleus ambiguus (2.12) and the hypoglossal nucleus (2.49). In contrast, the cerebellum (–0.02), spinal trigeminal nucleus (0.34) and area postrema (0.25) displayed low levels of specific binding. In general, this distribution is consistent with previous findings in rat brain (1). In the ferret brainstem (n = 3) a similar distribution of BHSP binding was observed. High binding was observed in both regions of the NTS (3.60 and 4.10, dNTS and vNTS, respectively), and also in the inferior olive (1.08), nucleus ambiguus (1.73) and the hypoglossal nucleus (1.41). As in the rat, low binding was observed in the cerebellum (0.13), spinal trigeminal nucleus (0.30) and the area postrema (0.69). The ability of SP (1 μM) and the NK_1 receptor antagonist CP 99,994 (CP, 1 μM) to inhibit BHSP binding to slices of both rat and ferret brainstem were also determined. These cold ligands completed for BHSP binding in most regions of rat brainstem with approximately equal potencies. However, a component of the BHSP binding in both regions of the NTS inhibited by SP was unaffected by CP. The NTS was subdivided in the coronal plane for further analysis. The resultant reduction in specific binding using CP was 26 and 23% (dNTS and vNTS, respectively, caudal region), 15 and 15% (postremal region) and 4 and 9% (rostral region). These data suggest that CP-resistant binding sites for SP exist in the rat NTS, predominantly located caudally. In contrast, there was no significant difference in the ability of SP and CP to inhibit BHSP binding in any areas studied in ferret brainstem. Electrophysiological studies have demonstrated an absence of NK_2 and NK_3 receptors in the rat NTS (2) and thus, it is possible that this CP-resistant site represents an atypical NK receptor.

1 Helke CJ *et al. Neuroscience* 1984; **12:** 215–223.
2 Maubach KA *et al. Can J Physiol* 1994; **72:** 460.

P23: The neurokinin$_1$ (NK_1) receptor antagonists CP-99,994 and RP67580 attenuate nicotine-induced emesis in *Suncus murinus*

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We have previously shown that the human type NK_1 receptor antagonist CP-99,994 (but not its enantiomer CP-100,263 which has a low affinity for the NK_1 receptor) inhibits emesis induced by cisplatin and apomorphine in ferrets (1, 2). The effects of these compounds and the rat-selective NK_1 receptor antagonist RP67580 have been tested against nicotine-induced emesis in *Suncus murinus* (house musk shrews). Nicotine was used as the emetogen, as apomorphine is not emetic in shrews.

Male shrews (55–80 g) were injected intraperitoneally (i.p.) with CP-99,994 (3 or 10 mg/kg), CP-100,263 (10 mg/kg), RP67580 (10 and 30 mg/kg), morphine (2 mg/kg: as a positive control) or drug vehicle (water) using a dosing volume of 0.2 ml/100 g body weight. Fifteen minutes later the shrews were injected subcutaneously in the scruff of the neck with nicotine (4 mg/kg). The number of retching and vomiting episodes occurring during the following 30 minutes was recorded.

Treatment	No. of episodes
Water + nicotine	19 ± 2
CP-99,994, 3 mg/kg + nicotine	9 ± 1 *
CP-99,994, 10 mg/kg + nicotine	3 ± 1 *
CP-100,263, 3 mg/kg + nicotine	18 ± 4
CP-100,263, 10 mg/kg + nicotine	17 ± 2
Water + nicotine	20 ± 3
Morphine, 2 mg/kg + nicotine	0 ± 0 *
Water + nicotine	19 ± 3
RP67580, 10 mg/kg + nicotine	19 ± 2
RP67580, 30 mg/kg + nicotine	2 ± 1 *

n = 4–8 * $p < 0.05$ Dunnett's test *versus* water pretreated shrews.

CP-99,994, RP67580 and morphine, but not CP-100,263, significantly antagonized the emesis induced by nicotine.

These results suggest that in the shrew, as in the ferret, NK_1 receptor antagonists are anti-emetic. There was only approximately a 3- to 10-fold difference in the dose of the rodent receptor selective RP 67580 when compared with the human receptor selective CP-99,994 giving a significant antagonism of emesis. This observation,

together with the lower activity of CP-99,994 in the shrew, when compared with the ferret (where a dose of 3mg/kg i.p. inhibits apomorphine-induced retching and vomiting) may reflect differences in drug pharmacokinetics and/or pharmacodynamics in this species. Alternatively, the NK_1 receptor in shrews may be neither rodent- nor human-like.

1 Tattersall FD *et al. Eur J Pharmacol* 1993; **250:** R5.
2 Tattersall FD *et al. Neuropharmacology* 1994; **33:** 259.

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